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BEET-MOSAIC VIRUS–GREEN PEACH APHID RELATIONSHIPS

EDWARD S. SYLVESTER[1]

(Accepted for publication Jan. 31, 1949)

The relationships between the non-persistent (6) viruses and their vectors have been the object of research of various authors (1, 2, 4). In several instances these relationships are well defined; *e.g.*, the influence of starvation upon vector efficiency, and the lack of prolonged retention. To date no critical data have been gathered upon such points as the acquisition and inoculation thresholds (3), the effect of short pre- and post-infection feeding starvation periods, and the influence of the number and duration of stylet insertions, or punctures, upon transmission. Data upon such points are presented in this paper.

MATERIALS AND METHODS

In the following experiments with the beet-mosaic virus, a total of 6 replications, 5 plants per treatment, for each variation were made. Single previously noninfective green peach apterae served as the vector, and they were transferred by the camel's hair brush technique. Where possible, a single virus source was used in each series of replications. The test plants were seedling beets in the cotyledon stage. In all cases where time was taken in seconds, a stop watch was used.

ACQUISITION THRESHOLD

The acquisition threshold refers to the minimum determinable time required by a feeding vector to acquire virus from a source plant. To determine the acquisition threshold of the beet-mosaic in sugar beets, using the green peach aphid as the vector, a series of 5-sec. interval infection-feedings was used. The first interval was a 5-sec. and the last a 30-sec. period. The data obtained in two separate tests are given in table 1.

The results of the two tests indicate that the acquisition threshold of this particular vector-virus association is between 6 and 10 sec. There appears to be an improvement in efficiency if the penetration is maintained for more than 10 sec., but not beyond 15 sec. Since test I suggested the possibility of either an exceptionally high value for the 15-sec. interval, or an exceptionally low value for the 20-sec. interval, a second test was made. Using the mean value from the two separate tests, it was concluded that feeding punctures maintained beyond the 15-sec. interval are of little value in improving efficiency.

Other results have indicated that a 5-min. infection feeding has no advantage over a 30-sec. infection feeding. Consequently, it appears that the first 15 sec. is the crucial period, as far as the length of infection feeding

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TABLE 1.—*Results[a] of trials to determine the acquisition threshold of the beet-mosaic virus, using starved single green peach aphid apterae, a variable infection feeding, and a 0.5- to 2-hr. test feeding period. In each test, a total of 6 replications, 5 plants per treatment, was used*

Test	Infection feeding period in seconds						Total
	5	10	15	20	25	30	
I	0/30	7/30	19/30	13/30	20/30	21/30	80/180
II	0/30	4/30	11/30	12/30	5/30	7/30	39/180
Mean	0/30	5.5/30	15/30	12.5/30	12.5/30	14/30	59.5/180

[a] In the ratios listed, numerator is number of plants infected; denominator, number of plants inoculated.

is concerned. This feeding period is only one of many factors which will influence the vector efficiency in any given test.

INOCULATION THRESHOLD

The trials for determining the inoculation threshold were done in the same manner as in the case of those of the acquisition threshold, with the exception that the infection feeding remained relatively constant, while the test feeding was varied. As in the acquisition threshold tests, the intervals used were 5 sec. apart, commencing with a 5-sec. test feeding and terminating with a 30-sec. period. The results of the trials are given in table 2.

The results indicate that the inoculation threshold lies between 6 and 10 sec. One difference in these data from those taken on the acquisition threshold was the lower level of transmission obtained in the inoculation threshold trials. Also there was little change in efficiency of transmission until the puncture of the test feeding period had been established for more than 25 seconds.

The lower level of transmission in the inoculation threshold trials apparently was a direct result of the activity of the vector on the test plant, since in the inoculation threshold test and the acquisition threshold test I replications, the virus source was common, as was the aphid colony. The test plants were taken from the same lot, and the comparative replications were done on the same day.

TABLE 2.—*Results[a] of trials to determine the inoculation threshold of the beet-mosaic virus, using starved single green peach aphid apterae, a single virus source, a 0.5- to 3-min. infection feeding period, and a variable test feeding period*

	Test feeding period in seconds						Total
	5	10	15	20	25	30	
Result	0/30	7/30	10/30	8/30	8/30	15/30	48/180

[a] In the ratios listed, numerator is number of plants infected; denominator, number of plants inoculated.

TRANSMISSION THRESHOLD

The term transmission threshold, which heretofore has not been defined, refers in this work to the minimum determinable period of time required for a single vector to transmit a virus from a disease source to a healthy plant. In the experiments to determine a transmission threshold value the following three variations were used: (1) an infection and test feeding period each 10 sec. in duration, (2) an infection and test feeding period each 15 sec. in duration, and (3) an infection feeding period of 15 sec. and a test feeding period of 20 sec. Again in this series of tests, 6 replications, 5 plants per treatment, for each of the 3 variations, were used. Only the data from the positive results are included in table 3.

TABLE 3.—*Results of test to determine the transmission threshold of the beet-mosaic virus using starved single green peach aphid apterae. The pre-infection feeding starvation period varied from 1.5 to 3.5 hr.; the infection feeding and test feeding intervals varied with the experiment. Only the data from the positive results are included*

IF: TF[a] (in seconds)			Results[b]	Transfer time[c]		Total time (in seconds)	
Intended	Actual	Mean		Range	Mean	Range	Mean
10: 10	10: 10	10: 10	4/30	23–87	42.25	43–107	62.25
15: 15	15: 10–15	15: 14.2	9/30	19–54	25.8	42–84	55.0
15: 20	15: 9–15	15: 16.8	10/30	15–37	23.8	45–67	55.6

[a] The IF: TF ratio expresses the second intervals that were used for the infection feeding and the test feeding periods, respectively.

[b] In the ratios listed under results, numerator is number of plants infected; denominator, number of plants inoculated.

[c] The term transfer time refers to the time elapsing between the termination of the infection feeding period on the virus source and the beginning of the test feeding period on the healthy plant.

The results obtained give a minimum value for transmission, or a transmission threshold, of 42 sec. The maximum in these trials was 107 sec., with a transmission threshold mean of 56.5 sec. Obviously the maximum value obtainable would vary considerably, depending upon the non-feeding activities of the individuals being used. The minimum value obtainable would be limited by at least three factors: (1) the time needed for attaining the acquisition and inoculation thresholds, (2) the time required by the manual act of transferring, and (3) the time consumed in an orientation period, apparently required by the insects after being transferred.

INFLUENCE OF THE LENGTH OF THE PRE-INFECTION FEEDING STARVATION PERIOD

In the initial experiment upon this phenomenon, the aphids were starved in a vial prior to the infection feeding for the following time intervals: 0, $\frac{1}{4}$, $\frac{1}{2}$, 1, 2, 3, 4, 5, and 6 hr. The infection feeding following starvation was approximately 30 sec., and the test feeding period was approximately 1 hr. in duration. The results of the trials are given in table 4.

TABLE 4.—*Results*[a] *of trials to determine the influence of the length of the pre-infection feeding starvation period upon vector efficiency. Single green peach aphid apterae fed, after the starvation period, upon a single virus source were used. The infection feeding was approximately 30 sec.; the test feeding approximately 1 hr.*

	Starvation time in hours									Total
	0	¼	½	1	2	3	4	5	6	
Result	3/30[b]	22/30	20/30	20/30	18/30	24/30	21/30	21/30	26/30	178/300

[a] In the ratios listed, numerator is number of plants infected; denominator, number of plants inoculated.

[b] The result is the mean of 2 replicated check series.

From the table it appears that the maximum effect of pre-infection feeding starvation can be obtained in a period of 15 min., and that little benefit is to be gained by increasing the length of this period to a matter of hours. Watson (5), using intervals of 0, 1, and 20 hr., obtained similar results.

Since the maximum effect obtained in the experiment was found in 15 min., a replicated series was done to test the intervals of time occurring between 0 and 15 min. The intervals used were: 0, 2, 5, 10, 15, and 60 min. The results are found in table 5.

In this case, the maximum effect of starvation was found to occur after 5 min. starvation, and but little, if any, increase was obtained for a 2-min. period. It appears that the effect of starvation upon the transmission efficiency occurs rather rapidly and suddenly, and if the action of any inhibitor or inactivator is responsible for the phenomenon, as has been suggested by Watson and Roberts (6), it is potent and rapid in its action.

INFLUENCE OF POST-INFECTION FEEDING STARVATION ON TRANSMISSION

Watson (5) reported that the beet-mosaic virus was lost most rapidly in the first few hours of post-infection feeding starvation, but that the transmission of beet-mosaic virus was less influenced by this treatment than were some of the other non-persistent viruses. Her evidence indicated that 22 per cent of the initially infective aphids were still infective after 20 hr. fasting.

TABLE 5.—*Results*[a] *of trials to determine the influence of the length of the pre-infection feeding starvation period upon vector efficiency. Single green peach aphid apterae, fed, after the starvation period, upon a single virus source were used. The infection feeding was approximately 30 sec.; the test feeding 1 hr.*

	Starvation time in minutes						Total
	0	2	5	10	15	60	
Result	2/30	4/30	16/30	15/30	17/30	14/30	68/180

[a] In the ratios listed, numerator is number of plants infected; denominator, number of plants inoculated.

TABLE 6.—*Results[a] of trials to determine the effect of post-infection feeding starvation on vector efficiency, using single green peach aphid apterae, single virus sources, a 0.5- to 3-min. infection feeding, a variable post-infection feeding starvation period, and a 1-hr. test feeding*

Environment[b] during starvation	Length of post-infection feeding starvation period in hours					Total	Unstarved check
	1	2	3	4	8		
Dry	3/30	0/30	0/30	0/30	1/30	4/150	9/30
Moist	4/30	0/30	1/30	0/30	0/30	5/150	.....

[a] In the ratios listed, numerator is number of plants infected; denominator, number of plants inoculated.

[b] The dry starved aphids were placed in a dry, filter paper-lined Petri dish; the moist starved aphids were placed in a moistened filter paper-lined Petri dish during the starvation period.

In testing this point, a replicated series of trials was made. The insects were starved prior to the infection feeding, given a 0.5- to 3-min. infection feeding, and then starved prior to being placed on the test plants for the following hourly intervals: 0, 1, 2, 3, 4, and 8 hr. The test feeding period was 1 hr. in duration. The results of the trials are given in table 6.

The results indicate that the initial rapid loss of the ability to transmit the virus under conditions of starvation is similar to those obtained by Watson (5). However, starvation beyond an hour resulted in almost complete loss of this ability. Watson starved the aphids under moist conditions, consequently table 6 includes aphids starved under dry conditions, and a comparable series starved under moist conditions, *i.e.*, in a Petri dish lined completely with moistened filter paper. The results indicate that the two environments used had little effect upon the results.

Since the loss of ability to transmit the beet-mosaic virus was so rapid during the first hour, trials were made to determine during what portion of the hour the loss was occurring. In table 7 are found the results of trials using the intervals of 0, 15, 30, 45, and 60 min. of post-infection feeding starvation.

The test indicated that the loss of the virus was most pronounced in the first 15 min. of post-infection feeding starvation. Consequently the

TABLE 7.—*Results[a] of trials to determine the effect of post-infection feeding starvation on vector efficiency, using single green peach aphid apterae, a single virus source, a 0.5- to 3-min. infection feeding, a variable post-infection feeding starvation period, and a 1-hr. test feeding*

	Length of post-infection feeding starvation period in minutes					Total
	0	15	30	45	60	
Result	13/30	6/30	2/30	1/30	0/30	22/150

[a] In the ratios listed, numerator is number of plants infected; denominator, number of plants inoculated.

next trial was made to test the intervals between 0 and 15 min. starvation. The intervals used in this test were: 0, 2, 5, 10, 15, and 60. Table 8 lists the data obtained on these intervals.

The results indicate that loss of the ability to transmit the virus can begin within 2 min. of post-infection feeding starvation, but that starvation from 2 to 15 min. is fairly uniform in its action.

Briefly the data indicate, when viewed as a whole, that there is an initial loss of ability of the green peach aphid to transmit the beet-mosaic virus, following 2 min. of post-infection feeding starvation. The degree of lost ability remains fairly constant for approximately 15 min., after which time it declines until about 1 hr. of starvation, at which time the loss is almost total. An occasional individual, however, may retain the ability to transmit the virus for as long as 8 hr., under conditions of starvation following an infection feeding.

TABLE 8.—*Results[a] of trials to determine the effect of post-infection feeding starvation on vector efficiency, using single green peach aphid apterae, a single virus source, a 0.5- to 3-min. infection feeding, a variable post-infection feeding starvation period, and a 1-hr. test feeding*

	Length of post-infection feeding starvation period in minutes						Total
	0	2	5	10	15	60	
Result	13/30	7/30	6/30	8/30	5/30	2/30	41/180

[a] In the ratios listed, numerator is number of plants infected; denominator, number of plants inoculated.

INFLUENCE OF THE NUMBER AND DURATION OF THE FEEDING PUNCTURES ON EFFICIENCY

Examination of the data which were obtained in the acquisition and inoculation threshold trials indicated that some factors were influencing the vector efficiency. Since, in the acquisition threshold trial I and the inoculation threshold test, a common virus source, aphids from one population, and comparable test plants were used in simultaneously run replications, it was probably something associated with the test feeding period that determined the level of transmission.

A test was set up which compared the level of transmission obtained when 1 stylet insertion or puncture of a specified duration was made upon a test plant, with the level obtained when 5 punctures, each of the specified duration, were made upon a test plant. The length of the individual test feedings was 10 sec. Individuals permitted a single 10-sec. test feeding inoculated 8 out of 30 plants, while those allowed 5 separate 10-sec. test feedings on a single plant inoculated 23 out of 30 plants. Thus the number of punctures made upon the test plant greatly influenced the transmission level. This was also true with a persistent aphid-borne virus, *viz.*, the

sugar beet yellow-net virus (3). In the case of the beet-mosaic virus, the vector efficiency apparently can be doubled, or possibly tripled, by increasing the number of punctures from 1 to 5. From this it follows that the longer the test feeding period, the more potential punctures can be effected, and thus one explanation can be made for the effects of long test feeding periods upon increasing vector efficiency.

The results obtained in the inoculation threshold trials indicated that after 25 sec. of feeding, a slight increase in transmission followed an increase in feeding time (Table 2). In order to test this factor further, another series of trials was designed which compared the vector efficiency of insects which were allowed a 10-sec. test feeding, with those allowed a 60-sec. test feeding period. In these trials, the individuals which were allowed a single 10-sec. test feeding period inoculated 2 out of 30 plants, while those permitted a single 60-sec. puncture inoculated 6 out of 30 plants.

There is a strong indication that the longer the stylets remain *in situ*, the greater is the amount of transmission. Considering the results of both the trials made by varying the number, and increasing the duration, of the stylet insertion or puncture, it appears that both factors influence vector efficiency.

CONCLUSIONS

The experimental results have suggested the following conclusions as to the transmission of the beet-mosaic virus by the green peach aphid.

The acquisition and inoculation thresholds lie somewhere between 6 and 10 sec., with a mean transmission threshold value of approximately 1 min. The pre-infection feeding starvation period, to be effective, must be between 2 and 5 min. Aphids starving following an infection feeding begin to lose their ability to transmit the virus within 2 min., but the loss appears to be greater after 15 min. of starvation.

When an infective insect is fed on a healthy plant, both the number of punctures that are made, and the duration of any single puncture, can affect the chances for inoculation.

From these results, it is apparent that any attempt to stop the field spread of such a virus should consider at least the following points: the degree of infestation, the normal activity of the insects involved, the type of population, especially in reference to the number of alate, or winged, forms present, and the potential results of any control program upon population movement and general restlessness.

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STUDIES ON OAK WILT, CAUSED BY CHALARA QUERCINA[1]

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(Accepted for publication January 10, 1949)

Oak wilt is one of the most destructive tree diseases. Large oak trees may be killed within a few weeks after the first appearance of wilt symptoms. In 1942 the disease was shown to be caused by a fungus (16), which was later described and named *Chalara quercina* Henry (8). Little is known, however, about the manner in which this pathogen invades and destroys oak trees so rapidly. The studies reported in this paper were undertaken to determine the route of parasitic invasion within infected oak trees, the mechanism of wilting, the influence of environmental conditions on development of the parasite, and means of preventing spread of the disease in state parks and forests of Iowa.

ECONOMIC IMPORTANCE

During the past five years oak wilt has become increasingly important in the Upper Mississippi Valley. The wilt pathogen has been isolated from wilting oaks in Wisconsin, Minnesota, Iowa, and Illinois (10), and the disease has been observed generally over the State of Iowa (5), in residential districts of St. Louis (2), and in other localities in Missouri (3).

Wilt is generally recognized as the most serious problem in oak culture in Iowa. Although the areas of infestation enlarge slowly, large numbers of oaks are killed each year because of the tremendous number of infested areas. In farm woodlots in northeastern Iowa sufficient trees were killed in 1945 (7) to constitute almost 70 per cent of the potential annual red oak growth in those areas. Many oak trees are killed each year in woodlots, parks, and forests in southwestern Wisconsin, southeastern Minnesota, and Iowa.

Apparently most North American species of oak are susceptible to wilt. Natural infection has been observed in Wisconsin (10) or in Iowa (4, 5, 7, 13) on *Quercus alba*, *Q. borealis*, *Q. coccinea*, *Q. ellipsoidalis*, *Q. imbricaria*, *Q. macrocarpa*, *Q. marilandica*, *Q. muehlenbergii*, *Q. palustris*, *Q. stellata* and *Q. velutina*. Many additional species have been shown by greenhouse tests to be susceptible to wilt: *Q. prinus* (4), *Q. bicolor*, *Q. montana*, *Q. rubra*, *Q. rubra* var. *pagodaefolia*, *Q. shumardii* (5), *Q. falcata*, *Q. garryana*, *Q. hemisphaerica*, *Q. laevis*, *Q. phellos*, *Q. shumardii* var. *texana*, *Q. suber* (1), *Q. gambelii*, *Q. laurifolia*, *Q. nigra*, and *Q. virginiana* var. *maritima* (7). No varieties tested have been resistant.

The disease is most severe on trees of the red oak group (7, 9, 10). Large trees may be dead within 4 to 6 weeks after symptoms first appear. Typi-

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cally, leaves in the crown of infected trees wilt, become bronze to brown, and fall from the tree. These symptoms appear progressively downward and inward throughout the tree. Red oaks infected in late summer or fall may put out leaves in the following spring, but develop typical symptoms and die within a few weeks. No red oak has ever been observed to recover from the disease. White and bur oaks are killed more slowly. Affected branches die, leaving many stagheads, but diseased trees may live for several years following initial infection.

No satisfactory method of controlling oak wilt has been found. Apparently the development of resistant varieties offers little promise. Host specificity does not seem to be an important factor, as limited tests showed that isolates from black, red, bur, and scarlet oak were pathogenic on black oak and an isolate from black was pathogenic on white oak (11).

Dietz and Barrett (6) reported that sanitary methods checked and sometimes controlled the spread of oak wilt. Tests (7) in which all infected oaks in an area and all healthy red oaks within a 50-foot perimeter were removed gave control of wilt. Results from plots in which only the dead and visibly diseased trees were removed were variable. In some plots spread was reduced, in others stopped. Pruning of infected branches was suggested as a means of saving white oaks with localized symptoms, although results were not perfect.

MATERIAL AND METHODS

Chalara quercina was isolated from naturally infected oak trees by the technique employed by Barrett (1). Sections of twigs, ½ inch or smaller, were surface-sterilized in a 20 per cent concentration of commercial Clorox for one minute and rinsed in sterile distilled water. The twig sections were then placed on a glass slide which had been dipped in alcohol and flamed, and serial cross-sections were cut with a flamed razor blade and planted on potato-dextrose agar. Larger branches with smooth bark were surface sterilized by drenching an area with alcohol and flaming. The outer bark was removed with a flamed scalpel and sections consisting of inner bark, cambium, and one- and two-year-old wood were removed and planted on potato-dextrose agar. On large branches and boles, with rough, cracked bark, the bark was split away from the wood with a wood chisel and pieces of one- and two-year-old wood were removed with a flamed razor blade. In all methods of isolation seven sections of tissue were placed on edge in potato-dextrose agar and incubated for 5 to 10 days at 20° to 25° C. Transfers were made to potato-dextrose-agar slants and the cultures were further purified by single endospore isolation, employing the method of Keitt (12), immediately before making the tests.

A special method of isolation was used for studying the distribution of the fungus within the bole of the tree. One-inch cross sections were cut at 8- to 12-foot intervals up the bole. A radial strip 2 inches wide and extend-

ing the complete diameter of the cross-section was taken from each disk. With a circular saw these strips were sawed half-way through at the cambium, between the second and third growth rings and at one-inch intervals across the remaining distance. The partially sawed sections of wood were easily broken off by inserting a wood chisel in the saw path and exerting lateral pressure, A portion of wood was cut from each exposed surface and placed on potato-dextrose agar.

Diseased tissues were studied by histological methods. Sections of diseased leaves, petioles, and young stems were killed in Craf III or FAA and dehydrated with normal butyl alcohol (14). The tissues were infiltrated with paraffin, cast in Parlax, and sectioned with a rotary microtome. Older stems were killed in FAA and sectioned with a sliding microtome. Satisfactory staining was obtained when the sections were mordanted in an aqueous solution of 1 per cent potassium metabisulfite and 1 per cent tannic acid for 30 minutes, stained for 30 minutes in hemalum, and counter-stained for one hour in safranin. When stained in this manner mycelial walls were dark red and protoplasmic inclusions were dark red to purple.

The effect of cultural conditions on spore germination was determined by observing germination on 1 per cent dextrose agar. Spores were obtained by incubating cultures on potato-dextrose-agar slants for 10 days at 20° C. The spores were washed from the surface of the slant in sterile distilled water and adjusted to a concentration of 50,000 spores per ml. A droplet of spore suspension (0.1 ml.) was placed at each of four loci on 1 per cent dextrose agar in a Petri plate. Observations on spore germination were made at intervals between 16 and 96 hours by counting about 100 spores from each plate. The spores were not washed prior to the tests since they consistently germinated better than 90 per cent, and the agar surface was used in preference to a hanging droplet because secondary endospores are typically produced from the germ tubes of germinating spores and confuse the count in liquid media.

Virulence of the cultures was determined by inoculation of 2- to 4-year-old plants grown in the greenhouse. Successful inoculations were obtained either by making a longitudinal slit in the bark, inserting a weft of mycelium and spores and wrapping with moist cheesecloth; by spraying a conidial suspension onto the tree and making punctures or longitudinal cuts sufficiently deep to introduce spores into the outer xylem; or by hypodermically injecting conidial suspensions into the outer xylem. Inoculated plants were kept in a moist chamber for 48 hours, after which time they were transferred to a greenhouse bench. Symptoms typically appeared on apical leaves after 10 to 20 days. No successful inoculations were obtained without wounding the host.

Field inoculations were made by removing a small (¾ by 1 in.) rectangle of bark and filling the opening with a mass of agar, mycelium, and spores. The opening was then covered with moist cheesecloth.

EPIPHYTOLOGY

To obtain information on the methods of overwintering and natural spread of the pathogen, detailed observations were made on field plots established at Dolliver, Call, and Pike's Peak State Parks and in State Forest areas near McGregor, Iowa.

Chalara quercina lived over winter commonly on white and bur oaks. Persistence of the pathogen in these trees for several years was shown by isolation from infected trees over a period of three years. Overwintering was less common on red oaks since they were killed quickly. The fungus was isolated readily from roots, boles, branches, petioles, and leaves of infected trees. The pathogen persisted in stumps for two years after infected red and white oaks were removed. Sprouts which grew from such stumps usually developed typical wilt symptoms by midsummer.

In order to obtain information on field spread, plots were established in isolated areas where one or only a few trees were infected. Plots were

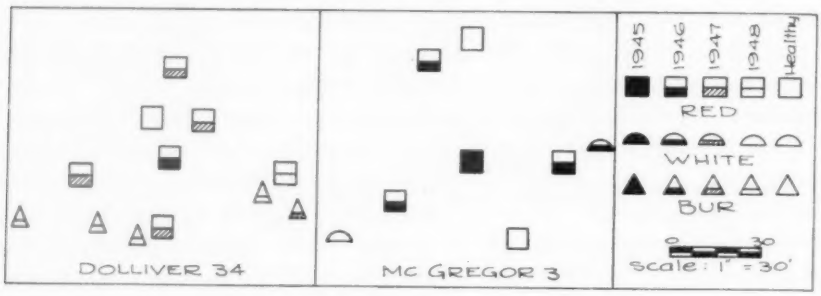

FIG. 1. Distribution of diseased trees in two localities within 3 years after initial infection. Each symbol represents one tree.

laid out with a rod and transit and maps were made of the location of all trees in each area. Records were taken periodically upon the amount of infection as determined by observation of symptoms and isolation of the pathogen.

Typical spread patterns are shown in figure 1. In evenly distributed oak stands spread usually occurred in a roughly concentric pattern from the locus of infection outward to the nearest surrounding trees. The average distance of spread from diseased to healthy trees was about 30 to 40 feet. Usually no infection was observed beyond those trees immediately surrounding the first point of infection. However, all trees within the range of spread were not always involved. In areas where the disease had been active for several years it was possible to observe the same slow outward pattern of spread by the relative deterioration of dead oaks.

These data do not coincide with the typical pattern of spread by airborne spores (15) or insects (17, 18). If infection were by either of those agencies it would be expected that the incidence of infection would vary

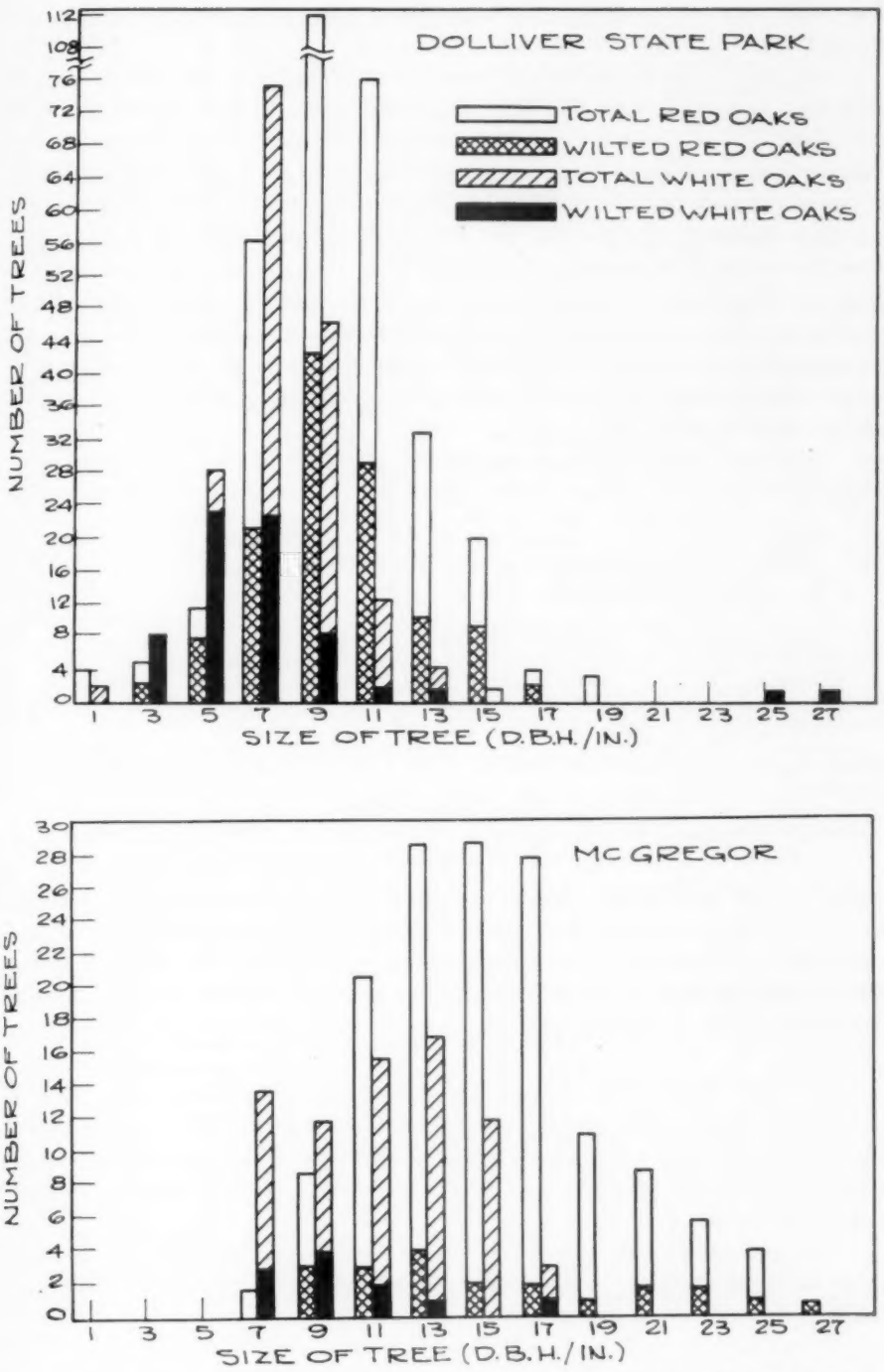

FIG. 2. (Upper) Total number of red and white oaks at plot 1, Dolliver State Park, and number of wilted oaks in each class. (Lower) Number of living red and white oaks of different sizes in plot 1, McGregor, in 1945, and number of trees in each class killed during 1946 and 1947.

inversely with the logarithm of the distance (18), and spread would be expected over a greater area than was observed.

Oak wilt is most obvious on large trees of the red oak group and it has been suggested that the large dominant trees in an area are the first attacked (5). To test for a correlation between size of tree and infection, complete surveys were made of all the oak trees in 8 wilt-infested areas. Typical data on the distribution of infection in two of these localities are given in figure 2. In general the number of infected trees of a given size was proportional to the total number of trees of that size in an area and the mean DBH (diameter at breast height) of the total population and of the wilted trees were almost identical. However, the mean DBH of the infected white oaks was slightly lower than the mean for the total white oak population. There is apparently no difference in the susceptibility of trees of different sizes to oak wilt.

To compare the incidence of natural infection of red and white oaks, data were tabulated on the percentage of the total volume of red and white

TABLE 1.—*Percentage of natural infection of red oak (Quercus borealis) and white oak (Q. alba) at four localities in northern Iowa, 1946–1947*

Location of plots	Red oak		White oak	
	Total no.	Percentage wilted	Total no.	Percentage wilted
Marquette Pt. I	60	10.0	89	6.7
Marquette Pt. III	39	17.9	41	2.4
Point Ann I	149	14.0	74	14.8
Dolliver I	90	11.1	50	12.0
Totals and averages	338	13.0	254	9.4

oaks infected in 1946–47 in areas in Dolliver State Park and near McGregor.

The data in table 1 show little difference in the susceptibility of red and white oaks to infection by *Chalara quercina* H. The extremely low figure for infection of white oaks in Marquette Point III is probably due to the unequal distribution of the species near the locus of infection in that plot.

At Pilot Knob State Park in north-central Iowa, dead and infected trees were so generally distributed throughout the park that study of spread from a single locus of infection was impossible. However, several areas were cruised and the number of dead and healthy trees of each oak species recorded (Table 2).

The percentages of the Hill's yellow (55.4) and red oak (52.9) trees that were dead were approximately twice as great as of white oak (28.3) and almost three times as great as of bur oak (19.9). However, large areas of dead bur oaks were found and numerous trees with a few dead branches were observed. These data bear out the observation that oak wilt is most destructive on species of the red oak group.

TABLE 2.—*Relative destruction of different species of oak at Pilot Knob State Park, Iowa, 1945*

Species	Total	Percentage dead
Q. macrocarpa (Bur)	2033	19.9
Q. alba (White)	300	28.3
Q. ellipsoidalis (Hill's yellow)	1688	55.4
Q. borealis (Red)	874	52.9
Total and average	4895	38.6

Records were taken on the time of first symptom development on trees in field plots at Dolliver State Park and McGregor, Iowa. The plots were checked at intervals of two weeks from May through September. During the summer of 1946 the greatest numbers of newly infected trees were recorded in June and July and a few were observed in May, August, and September (Table 3). In 1947, when the weather was subnormally cool

TABLE 3.—*Seasonal appearance of new wilt infection in field plots at Dolliver State Park and McGregor, Iowa*

Year of observation	Number of newly infected trees observed in				
	May	June	July	August	September
1946	5	30	29	5	5
1947	1	12	27	10	5

and heavy rains fell well into July, only a few trees had developed symptoms by the end of June and the peak of wilting was in July.

Host Specificity

Because of the wide variation in type of symptoms on different species of oak and the variation in rate of development in white oaks, tests were made for host specificity of different isolates. Cultures grown from single spores of isolates from six different species of *Quercus* were inoculated into

TABLE 4.—*Reaction of seven species of Quercus to Chalara quercina isolates from six different sources*

Species inoculated	Infection produced by culture from Quercus					
	borealis	alba	macrocarpa	ellipsoidalis	velutina	marilandica
Q. borealis	+	+	+	+	+	+
Q. ellipsoidalis	+	+	+	+	+	+
Q. palustris	+	+	+	+	+	+
Q. macrocarpa	+	+	+	+	+	+
Q. alba	+	+	+	+	+	+
Q. marilandica	+	+	0	+	+	+
Q. robur	+	+	0	+	+	+

+ = inoculation positive
0 = not tested

six healthy young trees of each of seven different species grown in the greenhouse. All isolates were pathogenic on all oak species tested (Table 4). The time required for symptom production and type of symptoms produced by each isolate was comparable. In addition to the species listed in table 4, numerous others were tested in the course of host range studies. No host specificity was observed in the different isolates.

DISTRIBUTION OF THE FUNGUS IN DISEASED TREES

The sudden and complete wilting of large oak trees suggests that *Chalara quercina* is a typical vascular parasite. If so, wilting might be induced by mechanical plugging of the vascular system, by production of a toxic substance which diffuses throughout the tree, or by rapid growth or transport of the pathogen from the site of infection throughout the tree. The distribution of the pathogen in five naturally infected red oaks was determined by host tissue isolations from all parts of the tree.

The pathogen was isolated from leaf midribs and petioles of wilting leaves, from branches and twigs bearing wilting leaves, and from the bole, but was not isolated from twigs on lower branches bearing apparently healthy leaves. The fungus was found well distributed perpendicularly in the bole but not always around the entire circumference. Radial distribution was restricted to the outer ¼ in. of sapwood. The fungus was never isolated from the heartwood.

Results of analyses of the distribution of the fungus within the tree by isolation were verified by study of microtome sections of diseased tissue from five red oaks and three white oaks. In these sections mycelium was observed in xylem vessels of leaf midribs, petioles, and twigs (Fig. 3). Mycelium was apparently confined to the xylem and was not present in all vessels. Most of the hyphae grew longitudinally in the vessels. Mycelial development was sparse in general but in a few vessels many hyphae were observed. Numerous conidia were observed in the vessels (Fig. 3, d). These could be distinguished readily from excised hyphal tips and sections by the bipolar droplets which were present in the conidia.

These analyses show that the pathogen was usually present in wilted leaves and in twigs and stems bearing wilted leaves. Thus wilting is not necessarily due to a toxin transported from other locations in the tree nor to mechanical plugging. The presence of the small (2 to 3×5 to 7μ) conidia in vessels would provide a mechanism for rapid dissemination of the fungus throughout the tree in the transpiration stream. Vessels of both red and white oak, even those in leaf veins, are large enough to permit passage of the spores.

In tests in which spore suspensions were forced through 12-in. sections of red, white, and bur oak twigs under pressure, spores were present in the first drops of liquid to be drawn from the twigs. Numerous chains of 8 to 10 spores passed through the vessels intact.

EFFECT OF CULTURAL CONDITIONS ON DEVELOPMENT OF THE PATHOGEN

Petri dishes containing potato-dextrose agar were inoculated with 0.05 ml. of spore suspension containing about 250,000 spores per ml. and incubated at 25° C. for 48 hours to allow equal germination in all plates. Four

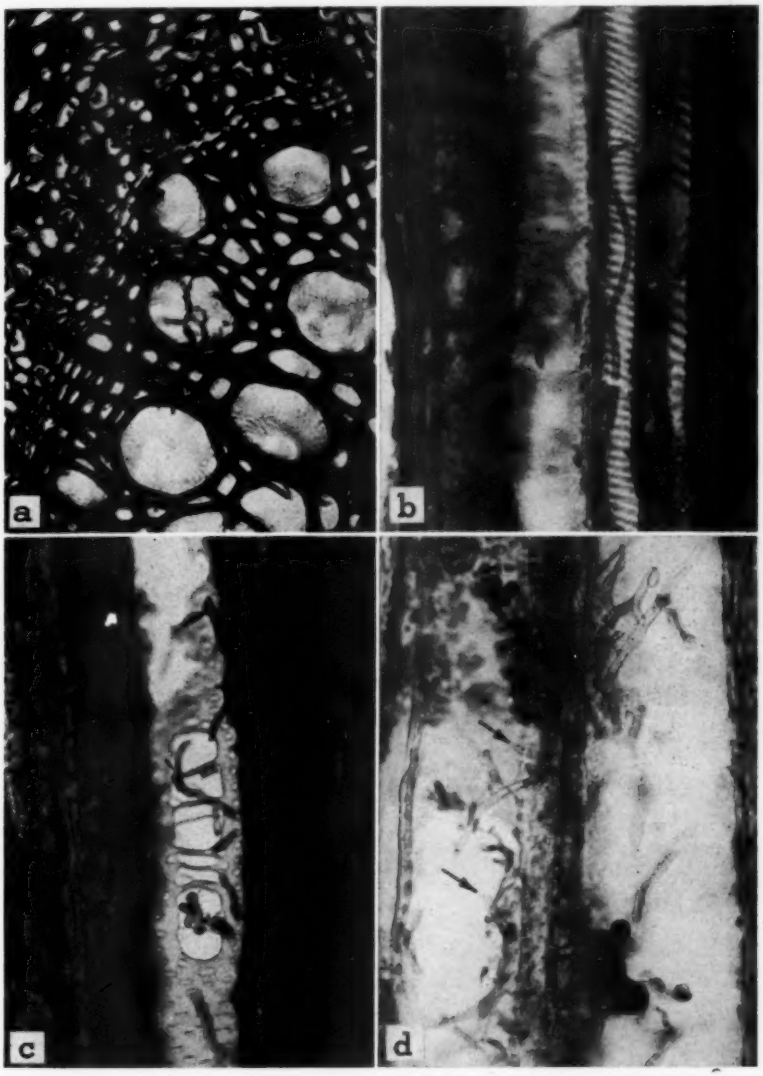

FIG. 3. Sections of diseased red oak showing Chalara mycelium in vessels. (317×) (a) Cross-section of petiole. (b, c, d) Longitudinal sections of current year's twig growth. Note endospores (arrows) in section d.

plates were placed in each of seven incubators adjusted to 5°, 10°, 15°, 20°, 25°, 30°, and 35° C. Daily growth increments were recorded by measuring the diameter of the colonies.

The most rapid growth (12 mm. per day) occurred at 20° to 28° C. No growth occurred at 35° C., and at 5° C. none occurred until after 10 to 14 days. Slow growth was recorded at 2° C. and 32° C. Growth at 10°, 15°, and 20° C. was linear with time. However, at 25° and 30° C. the curves showed a slowing of growth after the maximum rate was reached.

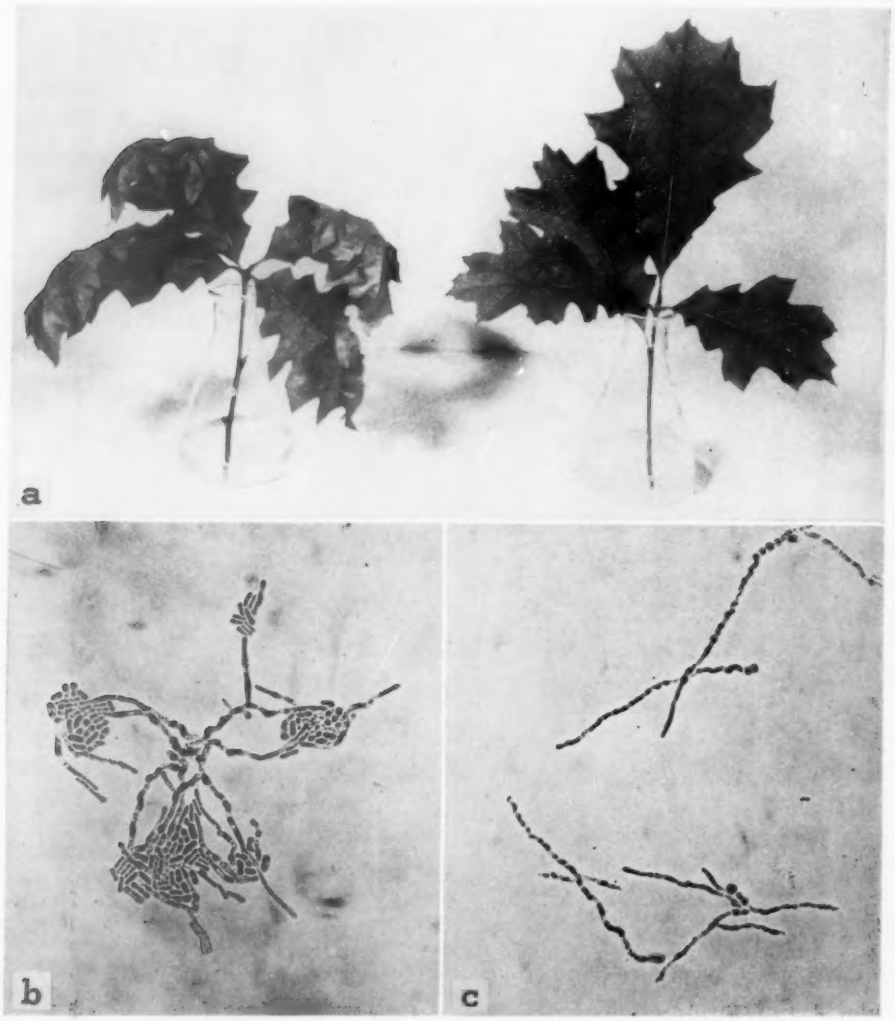

FIG. 4. (a) Red oak cuttings after 24 hours in culture filtrate (left) and uninoculated medium filtrate (right). (b) Production of endospores after 48 hours at 25° C. and (c) at 30° C.

This inhibition was probably due to the accumulation of toxic by-products of fungus metabolism. Similar observations were made on growth in liquid culture. Cultures in yeast extract solution were autolysed after 90 days at 25° C. Cultures of the same age maintained at 20° C. were still vigorous.

Spore germination tests were conducted over the same temperature range. Maximum spore germination occurred at 25° to 30° C. At this temperature 92 to 95 per cent of the spores germinated within 16 hours and up to 100 per cent after 24 hours. Germination was slow at the lower temperatures. At 10° C., 2 to 5 per cent germination was recorded after 96 hours. No germination occurred at 35° C. and spores which germinated at 32° C. did not continue to develop.

The method of germination differed markedly within the optimum temperature range. After 48 hours at 25° C. from 95 to 100 per cent of the germinating spores had produced numerous endospores (Fig. 4, b), the oldest of which had also germinated and were producing a second group of endospores. At 30° C. the majority of the germinating spores developed into vegetative hyphae and less than 15 per cent were producing endospores after 48 hours (Fig. 4, c). Endospores were produced abundantly after 48 hours at 20° C. and more slowly at 10° and 15° C. After 96 hours, spores had accumulated in large masses at 20° C. and 25° C. All spores that germinated at 15° C. were producing endospores, but at 30° C. endospore production was very limited.

Spore germination counts were made on plates of 2 per cent agar containing various concentrations of dextrose. Germination rose sharply from 0.0 to the 0.1 per cent level of sugar in the medium, with a range of better than 90 per cent at the 0.5 to 2 per cent sugar levels. From this point it tapered off to slightly better than 60 per cent at 4.0 per cent sugar. No higher concentrations were tested.

In addition to inhibiting spore germination, high concentrations of sugar were also unfavorable for the production of endogenous conidia by the germinating spores. The percentage of germinating spores that produced conidia from their germ tubes was lowest at the 4.0 per cent sugar level and increased as the sugar concentration decreased to 0.1 per cent. On the medium containing no sugar, sporulation was at a slightly lower level than on the 0.1 per cent medium.

Growth occurred over a range from pH 3.0 to pH 9.0 with the optimum from 5.0 to 7.0. The greatest amount of growth was recorded at 6.0. No growth occurred at pH 2 or pH 10.

MODE OF SYMPTOM INDUCTION

Spread of the pathogen through the host by movement of conidia in the transpiration stream, and induction of wilting by a metabolic product toxic to oaks, would explain the rapid progress of oak wilt through a tree. The rapid production of conidia by the pathogen and their movement through host vessels has been demonstrated.

To test for the production of metabolic products which would induce wilting, the fungus was grown in liquid culture on a modified Richards solution which contained asparagine and yeast extract. Culture filtrates

were obtained by passing the medium through a Büchner funnel and forcing the filtrate through a Seitz bacteriological filter to remove any Chalara particles or contaminating organisms. Dilutions were prepared by the addition of definite quantities of the filtrate to Erlenmeyer flasks containing measured amounts of sterile distilled water.

Oak cuttings placed in the filtrate reacted similarly to greenhouse-inoculated oaks. Severe wilting of young red oak cuttings was induced within 24 hours in a 50 per cent dilution of filtrate (Fig. 4, a). Wilting was general, with cupping and curling of leaves. After 70 hours large necrotic areas had developed between the veins. Bur and red oak cuttings showed slight symptoms of water loss after 24 hours, and after 120 hours were dry and papery and showed color zonation typical of that on leaves of greenhouse-inoculated plants.

The filtrates also were tested on 5- to 6-inch Bonny Best tomatoes. Plants were cut near the ground line with a razor blade, recut under water, and placed in the solution to be tested. In preliminary tests severe wilting of the cuttings was induced after 48 hours in 50 and 100 per cent concentrations of the filtrate. A small amount of drying of marginal lobes of leaflets was noted in control plants in noninoculated medium. Similar results were obtained using excised upper leaves from 10- to 12-inch plants.

To test for possible plugging of the lower stem, the tomato cuttings were removed from the filtrate after 48 hours, recut under water and placed in distilled water. None of the wilted plants recovered. Check plants treated similarly were still moderately turgid after 108 hours. Free-hand stem sections failed to show any indications of vascular plugging in the wilted plants. Bacteria were numerous in stems of the check plants and both cortical and vascular necrosis more extensive. It was also noted that control cuttings developed roots abundantly when recut and transferred to distilled water. Roots did not develop on the cuttings that had been exposed to the filtrate.

Dilutions of the filtrate ranging from 50 per cent down to 3 per cent were tested for toxicity to tomato cuttings. Severe wilting was induced within 48 hours in concentrations down to 12.5 per cent and slight marginal chlorosis and drying of leaflets was induced at 3 per cent concentration. Wilting was induced with filtrates from cultures varying in age from 15 to 90 days.

CONTROL

Although sanitation is usually more effective in control of canker diseases of trees than of wilts, studies were undertaken to determine whether infested areas could be disinfested by removal of diseased trees and infected white oak trees could be saved by removal of diseased branches. A series of plots established by Dr. S. M. Dietz was carefully surveyed for infection and records were taken on the progress of the disease before and after removal of diseased members.

Eradication experiments of three types were conducted. (1) In one area of heavy infestation all dead and diseased oaks and all living oaks within a 50-foot perimeter were cut in 1944. All branches, small twigs, and leaves were piled and burned and the stumps were stripped of bark. Logs and large limbs were sawed and used for firewood. No further spread was observed in this plot through 1947.

(2) In a second plot all dead and diseased trees and all living red oaks within a 50-foot perimeter were cut and disposed of as above. Living white and bur oaks were not removed from the plot. Trees continued to become infected in this area and were killed in large numbers in 1947.

(3) Two groups of plots separated by a valley one-half to three quarters of a mile wide were conveniently situated for a field comparison of the effect of eradication on incidence of infection, and were handled as follows. Spread was allowed to occur naturally in the 13 plots on one side of the valley from 1944 through the summer of 1945. In the winter of 1945–46 all dead and diseased trees were removed. A reduction in the number of infected trees of more than 50 per cent was observed in these plots during the summers of 1946 and 1947. In the second group of plots spread was allowed to occur without interference through the summer of 1946. The incidence of infection did not decrease in 1946. Following the removal of all dead and dying trees during November and December of 1946, however, a reduction of almost 40 per cent in the incidence of infection was noted in 1947.

From these data it appears that oak wilt may be controlled by thorough eradication of dead and diseased oaks in an area, provided a large enough perimeter of apparently healthy trees be removed from around the center of infection to eliminate any infected trees not showing symptoms. Failure to eradicate the pathogen from plot 2, and subsequent spread, were probably due to mildly infected white oak trees which went unnoticed in the removal of diseased trees. In all cases the yearly incidence of infection was decreased by removal of dead and diseased trees from infested areas. However, complete eradication was accomplished only in those areas in which a 50-foot perimeter of healthy trees was also removed.

The general distribution of the pathogen in infected red oaks precludes the use of pruning as a means of saving diseased trees of that group. However, oak wilt progresses slowly in white and bur oaks and apparently often is confined locally within the tree. If the latter were true, removal of infected portions might offer a satisfactory means of saving infected white oak trees. When the distribution of symptoms was favorable on naturally infected white oaks in field plots, diseased portions of the tree were removed at points 4 to 6 feet back of the innermost symptoms. In a few cases a portion amounting to as much as 15 to 20 feet of the tops of white oaks was removed.

The results of pruning were variable (Table 5). Apparently the organ-

ism was successfully eradicated from tree 7 by removal of four branches bearing wilted leaves. The remaining trees reacted variously in regard to recurrence of symptoms. Some developed general symptoms shortly after pruning. Others had no symptoms for over a year, then developed general symptoms.

TABLE 5.—*Effectiveness of pruning as a means of eradicating Chalara quercina from naturally infected white oaks*

Number and diameter of tree	Date of first pruning	Date symptoms recurred	Chalara reisolated	Remarks
1. 6-in.	June 20, 1945	July 15, 1945	Yes	General symptoms Cut July 15, 1945
2. 9-in.	do	July 18, 1946	No	
		June 13, 1948	Yes	Cut June 16, 1948
3. 11-in.	do	June 20, 1946	Yes	
		July 18, 1946	Yes	General symptoms
		July 12, 1947	Yes	Tree cut July 12, 1947
4. 9-in.	do	June 19, 1946	Yes	
		Sept. 18, 1947	Yes	Single branch pruned
		June 13, 1948	Yes	Single branch pruned
5. 7-in.[a]	June 21, 1946	July 12, 1947	Yes	General symptoms Cut July 12, 1947
6. 10-in.	July 10, 1946	July 12, 1947	Yes	General symptoms Cut July 12, 1947
7. 10-in.[a]	July 25, 1946	No symptoms	No	No further symptoms
8. 10-in.	July 13, 1946	July 27, 1946	Yes	General symptoms Cut July 27, 1946
9. 12-in.	July 11, 1946	Sept. 15, 1946	Yes	Pruned again
		June 20, 1948		General symptoms

[a] Symptoms scattered over tree. All branches with symptoms were pruned.

DISCUSSION

In the course of these investigations it has been observed that the oak wilt pathogen may live overwinter in infected trees and stumps. Trees of the white oak group may retain perennial infection for several years and red oaks may often harbor the pathogen for as long as a year. Definite proof has been offered that fungus mycelium and conidia develop in xylem vessels of these hosts. The manner in which hyphae or conidia leave infected plants and are transferred to healthy plants is not known. It is entirely possible that the fungus may have a perfect stage, as yet undiscovered, which would further supplement these known reservoirs of inoculum or which may be the chief means of dissemination.

Greenhouse studies indicate that the fungus cannot establish itself in uninjured stem or leaf tissue. No successful inoculations were obtained without first providing a wound entrance for the pathogen. Once established in the host, however, the organism spreads rapidly throughout the tree. The abundant production of small conidia provides a means of rapid spread in the transpiration stream. Conidia are produced most abundantly

at 20° to 28° C., a temperature range that prevails during the greater part of each summer day in June, July, and August in this region. Conidial production begins almost immediately following spore germination in culture and continues at a rapid rate as the fungus develops. Conidia may be found generally distributed in xylem vessels (Fig. 3, d) of infected trees.

Considering temperature as the chief factor limiting sporulation, the most favorable time for rapid spread of the organism would be in the latter half of June and first half of July when night temperatures are at a level permitting growth, and daytime temperatures seldom exceed the upper limits of the optimum range. Data on time of infection as based on development of symptoms bear out this hypothesis (Table 3).

The fact that conidia were readily passed through oak twigs under suction would indicate that they may be carried in the transpiration stream. The vessels of white oaks are smaller than those of red oaks, and more occluded with tyloses. Therefore, it is possible that these anatomical features may impede the movement of Chalara spores in white oak and in part contribute to the slower, more restricted, invasion of this type of oak.

The pathogen, when grown in liquid culture, produces certain metabolic products that are chemically stable and capable of inducing wilt symptoms typical of those associated with the disease. These toxic substances are not specific for oak since they also induce wilting of tomato cuttings in a similar fashion. The symptoms produced by the toxic products are so similar to the ordinary symptoms associated with oak wilt that there can be little doubt that the toxic substances play an important role in disease production. Red, white, and bur oak cuttings all reacted to the filtrate to produce symptoms typical of greenhouse-inoculated oaks. It is reasonable to assume, therefore, that the resistance in white oaks may be more appropriately attributed to restriction of invasion and growth of the fungus than to specificity of or immunity from the toxin.

Control of a tree disease such as oak wilt presents a tremendous problem. The sanitary measures which provided satisfactory disease control were too drastic to be of practical value for large-scale control efforts. Pruning of mildly infected white oaks may offer a means of saving individual trees. Uniformly good results from pruning elms with Dutch elm disease have been reported (18). Successful eradication of the pathogen from white oaks by pruning would depend on the reliability of symptoms as an indication of the extent of the pathogen's distribution within the tree. Preliminary results indicate that the organism is often restricted to the region of symptoms in white oaks.

SUMMARY

Chalara quercina was observed to overwinter commonly in white and bur oak trees, in stumps of diseased trees that had been removed, and occasionally in red oak trees that were infected late in the season.

The disease spread in a pattern not typical of windblown spore or insect dissemination. Oak wilt usually spread from an infection locus to the nearest healthy trees.

Data on percentage infection of red and white oak species showed little difference in susceptibility to infection. However, data on destruction of species of oak showed much greater losses in trees of the red oak group than in trees of the white oak group. No difference was observed in the incidence of infection of different sizes of trees. The number of infected trees of a given size was proportional to the total number of trees of that size in an area.

No host specificity was observed in cross-inoculation tests with isolates from different species of oaks. Isolates from each species produced typical disease symptoms when inoculated into other species.

Chalara quercina was isolated from all parts of diseased trees except the acorn. In histological preparations the fungus was observed in xylem vessels of leaf midribs, petioles, and stems. Conidia were observed in host tracheae, and conidia in water suspension passed readily through red and white oak stem sections.

Isolates of *Chalara quercina* grew well over a range from 16° to 28° C. with most rapid growth at 22° to 26° C. The optimum temperature range for spore germination was 25° to 30° C. At 25° C. endogenous conidia were typically produced from germ tubes of all germinating spores, while at 30° C. more than 85 per cent of the spores formed vegetative hyphae on germination.

The pathogen grew well on all complete nutrient media tested but grew best on potato-dextrose agar and oatmeal agar. Spores germinated best at 1 per cent dextrose concentration. Spore production from germinating spores tended to increase as the sugar concentration was decreased from 4 per cent to 0.1 per cent. The optimum pH range for growth was from pH 5 to pH 7 with limits at pH 3 and pH 9.

A metabolic product, which readily induced wilting of tomato and oak cuttings, was produced by *Chalara quercina* when grown on a modified Richards solution containing asparagine and yeast extract. Symptoms on toxin-wilted oak cuttings were typical of those produced on diseased greenhouse oaks.

Eradication experiments showed that the pathogen could be removed from an area by extreme sanitary measures. Incidence of infection was reduced by removal of diseased trees from areas of infection.

Results from pruning experiments were inconsistent but indicated that white oaks might often be saved by removal of diseased parts soon after the appearance of symptoms. Diseased branches were pruned at a point 4 to 6 feet back of the symptoms.

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PARASITIC ACTION OF STREPTOMYCES SCABIES ON ROOTS OF SEEDLINGS[1]

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INTRODUCTION

A sound approach to potato scab control by the use of suitable crop rotation is dependent, at least in part, on an understanding of the host range of the potato scab organism, *Streptomyces scabies* (Thaxt.) Waksman and Henrici.[3] Heretofore little attention has been given to the parasitism by *S. scabies* on the fibrous roots of various field crops, although infection of the roots of potato has been well established (4, 8, 13, 14). KenKnight (11) reviewed the literature describing scablike lesions on the underground organs of a large group of plants including beet, cabbage, turnip, rutabaga, radish, parsnip, and carrot. He reported as additional hosts, *Amaranthus retroflexus*, *Solanum nigrum*, and *Solanum melongena*.

The research herein reported was undertaken to determine the host range of *Streptomyces scabies* by investigating infection on the fibrous roots of various plants grown under a number of cultural conditions. It has been reported (6) from this laboratory that seedlings of various plants were unable to survive in peat soil artificially infested with *S. scabies*. Additional progress reports (5, 7) concerning certain phases of the work have been made.

EXPERIMENTAL PROCEDURE

Seed of plants to be tested for resistance to *Streptomyces scabies* were surface disinfected in 25 per cent commercial Clorox (5.25 per cent sodium hypochlorite) for 15 min.[4], rinsed in tap water, and germinated on sterile moist filter paper in Petri plates. When the radicles were approximately 1 cm. long, plants were transferred to the respective media for growth.

In the early trials, sterilized peat soil contained in $\frac{7}{8}$-in. Pyrex test tubes which were either 8 or 12 in. long was artificially infested with cultures of *Streptomyces scabies*. After approximately 1 week of incubation, a germinated seed was planted in the soil of the tube and the cotton plug was replaced. Controls containing sterilized, noninfested soil were maintained. In other trials, seeds were planted directly into pots containing sterilized peat soil which had been artificially infested.

Root infection in quartz sand cultures was obtained by mixing a suspension of spores and aerial hyphae from a culture of *Streptomyces* sp.

[1] Journal Paper No. J-1560 of the Iowa Agricultural Experiment Station, Ames, Iowa. Project 816. Supported in part by Bankhead Jones funds.

[2] Research Assistant Professor of the Botany and Plant Pathology Section, Iowa Agricultural Experiment Station, Ames, Iowa.

[3] Binomials of microorganisms used by Breed *et al.* (3) are employed.

[4] Although this treatment reduced the number of microorganisms on the surface, bacterial contaminants were often demonstrated when seedlings were planted on potato-dextrose agar.

with sterilized sand contained in either 4-in. pots or 250-ml. beakers equipped with suitable drainage tubes. Germinated seed were covered with infested sand and the sand was kept moist with tap water. After approximately 10 days' growth, plants were watered with a modified Hoagland's solution (20).

Inoculum was grown on modified potato-dextrose agar (19) in 9-cm. Petri plates. It consisted of a suspension of aerial hyphae and spores from $\frac{1}{3}$ to $\frac{1}{4}$ of a Petri dish culture, and was poured into a covered sterile 100-ml. beaker. This inoculum was mixed with sterile, melted (37°–40° C.) soil agar prepared by extracting the water-soluble material from 25 gm. of soil in 1 liter of water and solidifying the filtrate with 1.3 per cent agar. After the infested medium had solidified, radicles of germinated seed were inserted. Lids were removed in a day or two to permit growth of aerial portions, and tap water was used for watering.

Plants were grown at room temperatures (20° to 25° C.) and under continuous illumination. After the growth period, roots were readily washed free from the agar and fresh root weights were recorded. Preliminary trials with wheat and pea had shown that differences in fresh root weights were similar to those obtained with dry root weights and, because of ease of preparation, the former were used.

Four cultures of *Streptomyces scabies* were compared with six of *Streptomyces* sp. which were incapable of causing scab on tubers of Cobbler potato (*Solanum tuberosum* L.). A brief description of these ten cultures is given in table 1. The cultures were tested on soybean (*Glycine max* Merr. var. Lincoln), pea (*Pisum sativum* L. var. Little Marvel), wheat (*Triticum vulgare* Vill. var. Cadet Spring), radish (*Raphanus sativus* L. var. Early Scarlet Globe), garden beet (*Beta vulgaris* L. var. Detroit Perfected Strain), corn (*Zea mays* L. inbred Hy), and cucumber (*Cucumis sativus* L. var. National Pickling).

For histological studies, plants were grown in 2 per cent soil agar. Tissues were killed in Navaschin's Craf III solution (18), dehydrated in dioxan and N-butyl alcohol, imbedded in paraffin and cut at 15 μ. Sections were stained in hematoxylin for 12–16 hr. preceded by 4–8 hr. in iron-alum mordant. Although results with hematoxylin were very satisfactory, safranin was occasionally used as a counter stain.

INFECTION IN ARTIFICIALLY INFESTED PEAT SOIL

Seedling plants grown from surface disinfected seed were transplanted to large test tubes containing sterilized peat soil infested with *Streptomyces scabies*. Infection of aerial parts was common, especially in cucumber with aerial *Streptomyces* filaments present on either the cotyledons or growing point. Other plants responded by a general collapse of aerial portions accompanied by severe root necrosis (Fig. 1). Plants grown in the presence of *S. scabies* died prematurely, whereas those grown in sterilized, noninfested peat survived to the end of the observation period (Table

TABLE 1.—*Source and certain characteristics of the Streptomyces culture tested*

Culture number and name	Source of isolate[a]	Ability to scab Cobbler potato	Growth on soil agar	Color of surface growth on modified potato-dextrose agar[b]	Color of surface ring in separated milk[b]
1. *Streptomyces lavendulae* (Waksman and Curtis) Waksman and Henrici	S. A. Waksman	none	trace	pale mouse gray	Brussels brown
2. *S.* sp.	C. H. Meredith (culture 216)	none	slight	pale purplish gray	clear
3. *S.* sp.	shallow scab lesion on potato tuber	none	slight	white	clear
4. *S. scabies*	do	moderate	trace	pale gull gray	Brussels brown
5. *S. scabies*	deep scab lesion on root of red beet	marked	trace	pallid purplish gray	Brussels brown
6. *S.* sp.	do	none	slight	light gull gray	clear
7. *S. scabies*	do	marked	trace	pallid purplish gray	Sudan brown
8. *S.* sp.	apparently scab free skin of potato tuber	none	slight	pallid neutral gray	Brussels brown
9. *S. scabies*	shallow scab lesion on potato tuber	marked	slight	drab gray	Brussels brown
10. *S.* sp.	do	none	slight	white	clear

[a] Cultures 3, 4, 8, 9, and 10 were isolated in February, 1944, and cultures 5, 6, and 7, in September, 1944, from plants grown in peat soil at Crystal Lake, Iowa.

[b] Colors according to Ridgeway (16).

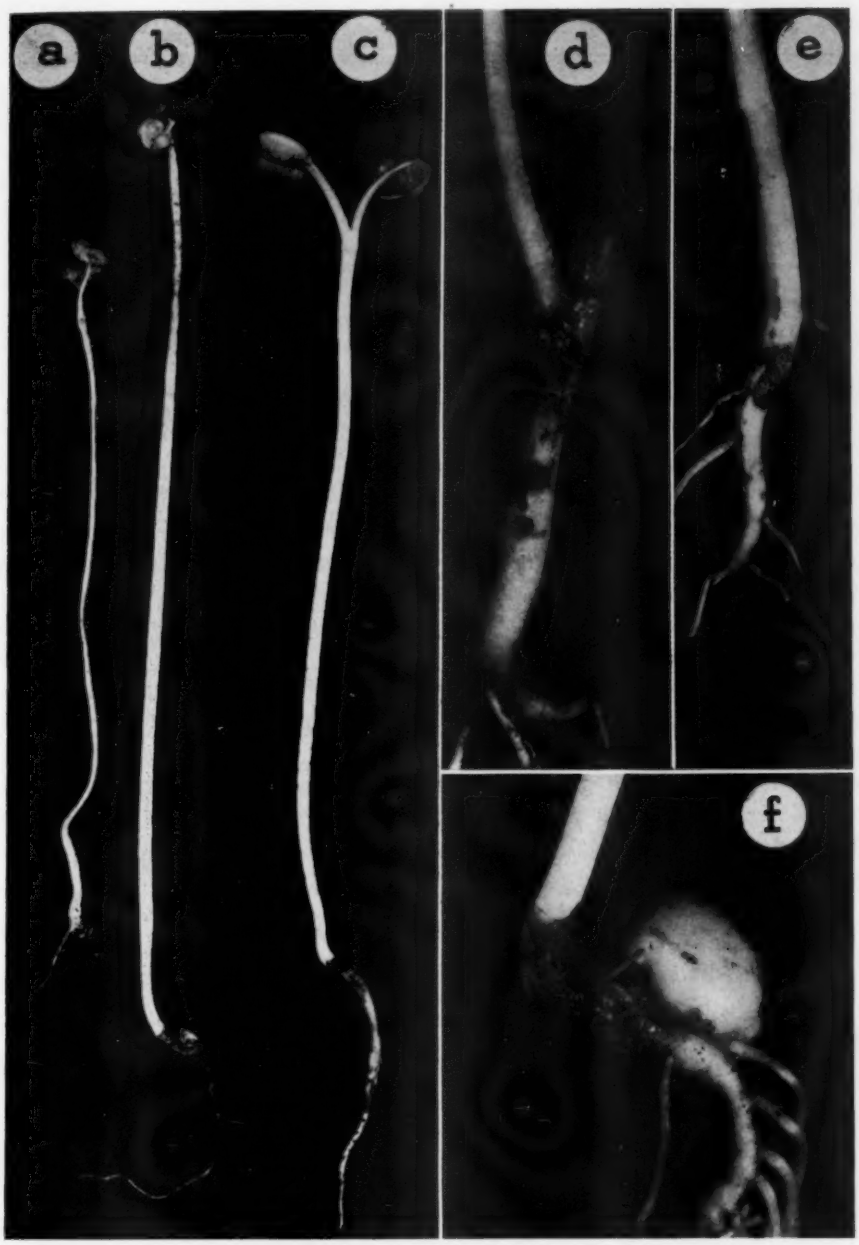

FIG. 1. a, b, c. Radish seedlings grown 16 days in tubes containing peat soil, (a, b) infested with *Streptomyces scabies*, and (c) noninfested. d, e. Lesions on soybean grown in pots containing peat soil infested with *S. scabies*. f. Lesion on pea grown under similar conditions. *Streptomyces* filaments were demonstrated in lesions of d, e, and f. (Photographs by John Stabe.)

TABLE 2.—*Survival of seedlings grown in large test tubes containing sterilized peat soil artificially infested with Streptomyces scabies*

Test plant	Period of growth	Infested peat[a]		
		Healthy	Diseased	Dead
	Days	*Number*	*Number*	*Number*
Radish, Scarlet Globe	19	0	2	11
	14	0	1	4
Turnip, Purple Top White Globe	22	0	3	3
	22	0	1	3
Cabbage, Improved Wis. Hollander	30	0	0	5
Spinach, Nobel Giant	11	0	2	4
	15	0	4	2
Cucumber, Early Pickling	18	1	7	2
	23	0	4	3
Potato, 116–13, open pollinated seed	19	0	1	5

[a] Equal numbers of control plants in noninfested peat remained healthy in each case.

2). In spite of the precautions taken to develop plants under aseptic conditions, it was in only rare instances that bacterial contaminants were not demonstrated when transfers were made at the end of the growth period to potato-dextrose agar. Although *S. scabies* was not in pure culture, the reaction was consistent with plants developing well in the noninfested controls and with necrosis and premature death where *S. scabies* was present.

Attempts to develop infection in open pots in the greenhouse were generally unsatisfactory, although scablike lesions were occasionally obtained on the lower stems of garden pea and soybean plants grown in infested peat (Fig. 1). In these lesions, filaments of *Streptomyces scabies* were demonstrated histologically in the tissues and spores and aerial filaments were present on the surface of lesions.

INFECTION IN QUARTZ SAND

Surface disinfected wheat seed were planted in sterilized, infested

TABLE 3.—*Necrosis of wheat root tips in quartz sand artificially infested with Streptomycetes*

Culture number	Pathogenicity to potato	*Experiment 1*				*Experiment 2*	
		13 days' growth		23 days' growth		23 days' growth	
		Roots[a]	Necrotic at tip	Roots[a]	Necrotic at tip	Roots[b]	Necrotic at tip
		Number	*Number*	*Number*	*Number*	*Number*	*Number*
4	moderate	39	9	40	12	38.2	26.2
5	severe	40	17	38	30	.....	.....
9	severe	44	17	41	36	34.5	31.7
6	none	36	0	42	0	34.3	0.3
none	.....	45	0	39	2	37.2	0.2

[a] Figures indicate the total number of seminal roots on 10 seedlings.
[b] Figures represent the average of 4 replications of 10 seeds each.

quartz sand. After 2 to 3 weeks, symptoms on wheat consisted of an orange-yellow to light brown necrosis of the root tips with generally no macroscopically visible discoloration of the cortex. The extent of root tip necrosis in the first trial (Table 3) became progressively more severe from 13 days to 23 days with cultures 4, 5, and 9. In a more extensive test, the extent of lateral branching was slightly reduced with cultures 4 and 5, pathogenic to potato, and root tip necrosis was similar to that previously obtained. In this second trial there was only a trace of root tip discoloration, the cause of which could not be determined, in the water controls and with culture 6.

Quantitative measurements of root necrosis with soybean were difficult since a light tan cortical discoloration was present over much of the root

TABLE 4.—*Growth of seedling plants in soil agar infested with Streptomyces sp. cultures*

Culture number and species	Weight of roots grown in infested soil agar[a]						
	Soybean	Pea	Wheat	Corn	Cucumber	Radish	Garden beet
	mg.	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*
4. *Streptomyces scabies*	152	90	28	279	73	38	4.8
5. do	109	97	16	268	84	31	5.3
7. do	104	89	18	250	82	22	6.3
9. do	131	97	12	263	92	27	6.6
Av.	124	93	18	265	83	30	5.7
1. *S. lavendulae*	268	363	34	343	99	49	9.1
2. *S. sp.*	309	355	47	396	100	67	9.6
3. do	344	334	45	416	97	52	7.1
6. do	392	373	45	466	95	46	7.2
8. do	273	270	51	344	81	53	8.6
10. do	253	333	46	366	80	56	8.0
Av.	290	339	45	389	92	54	8.3
Water control	318	353	51	381	78	56	9.0
do	306	.....	49	386	80	66	.....
Av.	312	353	50	384	79	61	9.0

[a] Figures represent the average fresh weight of roots from at least 3 beakers each containing approximately 5 plants of soybean, wheat, radish, and beet, and 3 of pea, corn, and cucumber. Growth period, 16 days except 10 days with soybean and 18 days with corn and cucumber.

area as well as at the tips. In type it was similar to that observed later in the soil agar, but it was not so consistently severe.

INFECTION IN SOIL AGAR

In December, 1945, experiments were begun using soil agar as a medium for plant growth. Such a medium permitted observation of roots during the growth period and supported only meager growth of the streptomycetes under observation (Table 1). Contaminants developed only sparsely in it.

Growth responses of soybean, pea, wheat, corn, cucumber, radish, and

garden beet to the ten cultures in soil agar are shown in table 4. Each of the plants listed was tested at least three times, and average root weights are presented from one representative trial consisting of at least three similar beakers, each containing equivalent numbers of plants. With all plants except cucumber, cultures fell into two distinct groups when separation was made either on the basis of root weight or of root necrosis. *Streptomyces scabies* cultures 4, 5, 7, and 9, known to cause scab on potato, reduced the root growth and severely impaired the development of laterals. Root necrosis was severe, especially at the tips and at the bases of the secondary roots. Root growth with cultures 1, 2, 3, 6, 8, and 10, known to be nonparasitic to potato, was neither appreciably reduced nor different from that developing in the sterile soil agar controls. Cucumbers had considerable resistance to *S. scabies* with little root weight reduction at the end of 18 days' growth.

Soybean and pea. These plants exposed to the four cultures of *Streptomyces scabies* had similar symptoms, generally becoming evident in 6 days, characterized by severe necrosis of the root tips and a severe general cortical necrosis of both the primary and lateral roots, with the latter being either absent or very short (Fig. 2). Fresh root weights (Table 4) with *S. scabies* were less than one-half those of roots grown in the water controls or with cultures nonparasitic to potato. Roots exposed to cultures nonparasitic to potato were white, well branched, and generally indistinguishable from those of plants grown in the water controls. Although there was some variation in root weights of these plants within the group of cultures nonparasitic to potato as well as within the group of *S. scabies* cultures, no definite trend was evident in subsequent trials. There was no overlapping of either root weights or necrotic reaction between cultures of either group. Aerial portions of soybean and pea were usually stunted, reflecting the severely necrotic condition of the roots. In certain instances aerial portions were slightly swollen, suggesting systemic invasion by *S. scabies*. This reaction was especially common when sprouted seeds were planted somewhat too deeply in the agar.

Henderson's bush Lima bean (*Phaseolus lunatus* L.) was similar in reaction to soybean and pea.

Small grains. Cultures of *Streptomyces scabies* caused necrosis of wheat root tips which was usually evident by the sixth day as a light tan discoloration, later changing to various shades of yellow to orange and light brown, and in advanced cases to dark brown. Generally there was little to no discoloration of the root cortex away from either the tip or points of origin of lateral roots (Fig. 3). After a root tip had become necrotic no further elongation was observed. Lateral roots generally failed to emerge, but their position was evident as punctate necrotic spots located at regular intervals along the roots. It was demonstrated histologically that these root tips had been almost replaced by a mass of streptomycetous filaments. Fresh root weights with *S. scabies* (Table 4) were less than one-

half those of plants grown in the controls or with the cultures nonparasitic to potato.

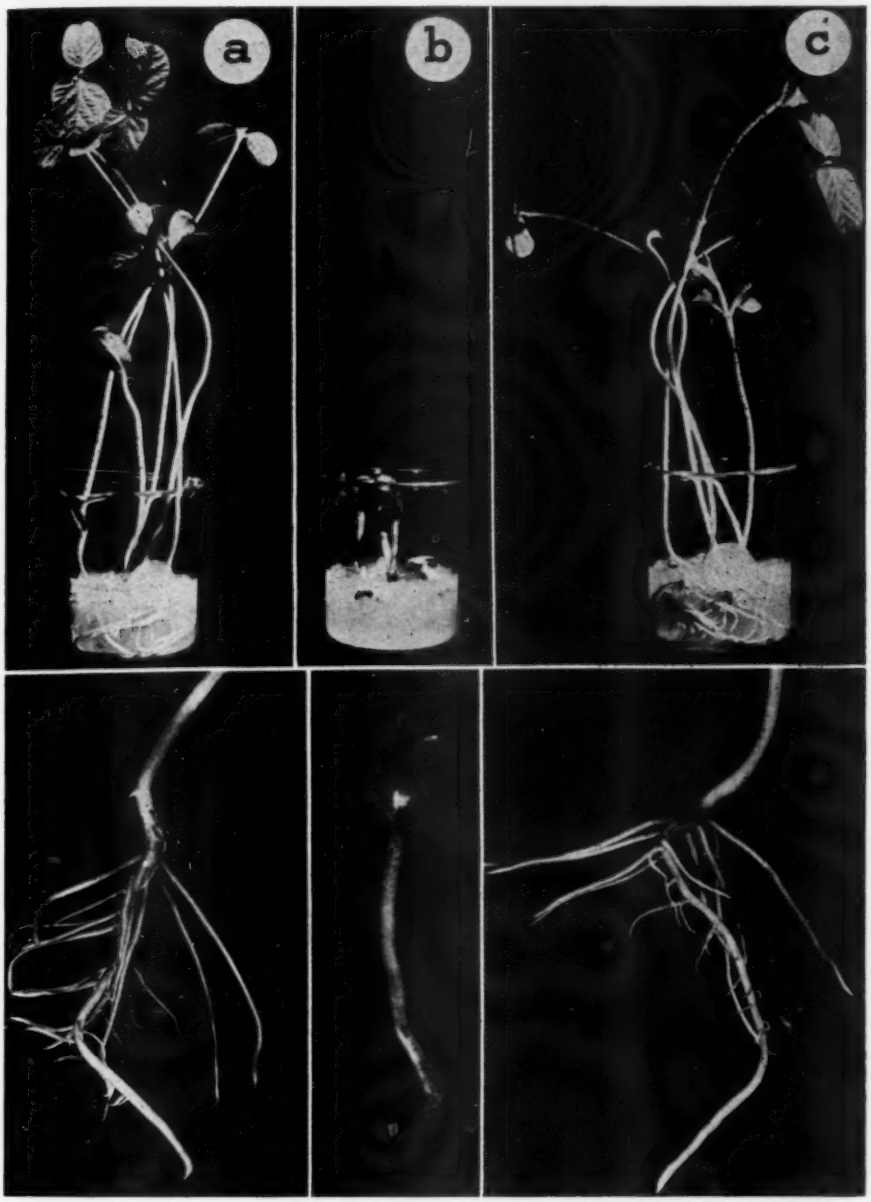

FIG. 2. Soybeans and representative roots grown 10 days in beakers containing soil agar: a, infested with culture 6 of *Streptomyces* sp.; b, infested with culture 7 of *S. scabies*; c, water control.

Root growth of control plants in the agar medium alone was generally good with lateral roots and root hairs present by the fifth day and with no

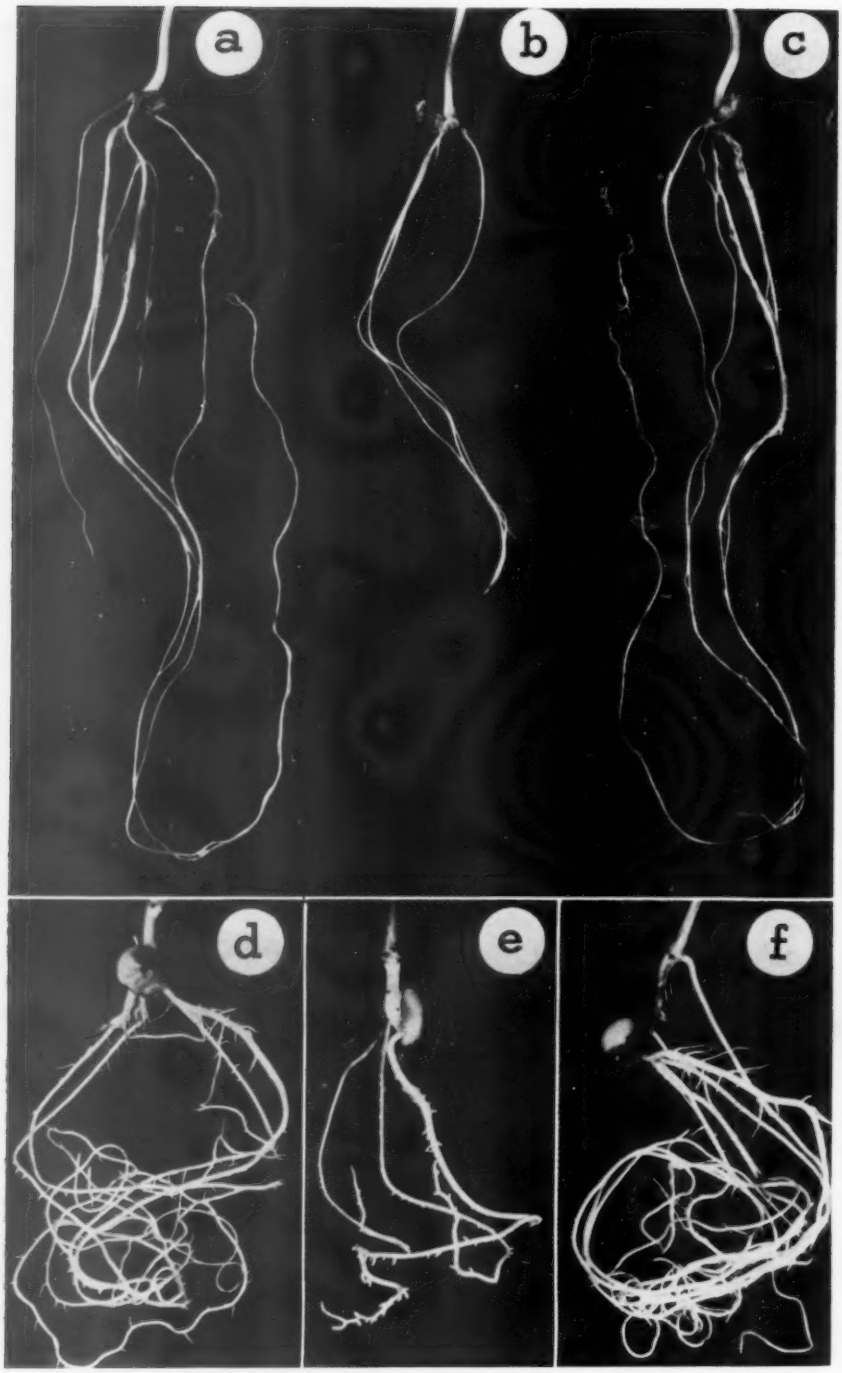

FIG. 3. (a, b, c) Wheat roots grown 14 days, and (d, e, f) corn roots grown 18 days in soil agar: a, d, infested with culture 6 of *Streptomyces* sp.; b, e, infested with culture 7 of *S. scabies*; c, f, water control.

necrosis evident throughout the period of observation. Roots were similar in both the water control and cultures nonpathogenic to potato. However, root hair development in cultures nonparasitic to potato was variable. Certain cultures seemed to have no deleterious effect on root hairs, whereas with other cultures root hairs were very sparse or lacking. The relative abundance of root hairs was not noticeably correlated with either root or top growth.

Other species of small grains were tested with representative cultures. The following were found to be as susceptible to *Streptomyces scabies* as wheat: *Avena strigosa* Schreb.; *A. byzantina* C. Koch var. Bond; *A. sativa* L. var. Markton; *Hordeum vulgare* L. (winter and spring types); *H. nodosum* L.; *H. distichon* L. var. Spartan; *H. bulbosum* L.; *Triticum vulgare* Vill. var. Mida and an unnamed winter type; *T. durum* Desf.; *T. timopheevi* Zhuk.; and *Secale cereale* L. var. Balbo.

Corn. Plants after 12 days' growth with cultures nonparasitic to potato and in the water controls produced slender fibrous roots, whereas roots with cultures of *Streptomyces scabies* were somewhat shortened and thickened. Average root growth with *S. scabies* after 18 days was reduced (Fig. 3) to 69 per cent of either the water controls or the cultures nonparasitic to potato. Lateral roots were only slightly discolored without advanced necrosis with cultures of *S. scabies*, whereas there was no necrosis in the water controls or with cultures nonparasitic to potato. When plants were grown for 32 days, marked necrosis was evident at the root tips and the points of emergence of secondary roots, suggesting that corn was not so susceptible as wheat, pea, and soybean to *S. scabies*.

Cucurbitaceae. Of the plants tested, seedlings of cucumber and cantaloupe (*Cucumis melo* L. var. Golden Fleshed Honeydew) were the most resistant. After 18 days (Table 4), there was no appreciable difference in the type or amount of cucumber root growth in any of the cultures or in the water controls. No necrosis was evident in any culture at this time. In another test, in which plants were grown for 35 days, although roots showed little to no necrosis with *Streptomyces scabies*, they were only 72 per cent as heavy as those grown with cultures nonparasitic to potato. When the tops were accidentally injured mechanically, root necrosis promptly developed.

Roots of *Cucurbita pepo* L. var. Acorn Squash exposed to *Streptomyces scabies* were slightly necrotic in two of three trials, whereas those of *C. moschata* Duchense var. Large Cheese Pumpkin had little to no root discoloration.

Other plants. A number of small seeded vegetables and legumes, although unrelated taxonomically, had similar symptoms. Radish roots grown in the presence of *Streptomyces scabies* were necrotic at the tips and at the points of origin of laterals. Root weights (Table 4) were approximately one-half those in the water controls or with *Streptomyces* sp. nonpathogenic to potato. Where *S. scabies* was absent, plants grew well,

producing abundant lateral roots with no necrosis. As in pea, aerial parts were often stunted and hypertrophied, suggesting systemic invasion by *S. scabies*. Garden beet (Table 4) was similar to radish, except that the necrosis was extremely severe and lateral roots were almost completely inhibited.

Although onion (*Allium cepa* L. var. White Spanish) did not grow well in the soil agar medium, roots were necrotic indicating susceptibility. Tomato (*Lycopersicon esculentum* Mill. var. Pan American), although very susceptible, was slightly more tolerant than eggplant (*Solanum melongena* L. var. Black Beauty). Carrot (*Daucus carota* L. var. Imperator), parsnip (*Pastinaca sativa* L. var. Hollow Crown), and lettuce (*Lactuca sativa* L. var. New York No. 12) were susceptible.

Alfalfa (*Medicago sativa* L.), although not immune, was slightly more resistant than red clover (*Trifolium pratense* L.), alsike clover (*T. hybridum* L.), and sweet clover (*Melilotus alba* Desr.).

Seedlings grown from open pollinated seed of a number of potato varieties and progenies were tested in soil agar for resistance to root necrosis. Seedlings from scab resistant progenies, Cayuga, 116–16, 116–13, and 528–194, were apparently no more resistant than seedlings from susceptible Cobbler and Teton, although infection of cotyledons may have been a factor in the final reaction.

HISTOLOGICAL STUDIES OF PLANT ROOTS GROWN IN AGAR

Histological preparations were made to determine the relationship of the potato scab organism to plant roots developing in infested agar[5]. Blocks of agar through which wheat roots had grown were placed in killing solution ten days after the germinated seed had been planted. At this time necrosis was beginning on the root tips, but the main portions of the root appeared superficially to be unaffected (Fig. 4, A, B). Filaments of *Streptomyces scabies* were present in most of the epidermal cells and in certain of the cortical cells. Five blocks were examined from various parts of the beaker. In each case, even though the root was not visibly necrotic, streptomycetous filaments were observed in the epidermal cells. Radish roots grown eight days under similar conditions were sectioned and the organism was likewise demonstrated both in the cortical and epidermal cells. *S. scabies* culture 7 was grown on a thin agar film on a glass slide and killed, stained, and dehydrated in the usual manner. Filament diameters were similar to those in the infected cells of wheat and radish roots. No filaments were observed in wheat or radish epidermal cells with *Streptomyces* sp., culture 6, under similar conditions.

Growth of *Streptomyces scabies* was consistently stimulated in the immediate vicinity (rhizosphere) of both wheat and radish roots. Abundant filaments in the vicinity of the root were observed in temporary mounts prepared by placing blocks of agar, through which a root had grown, in

[5] The author is indebted to Dr. John E. Sass of the Department of Botany, Iowa State College, for advice and very helpful cooperation in this phase of the work.

very dilute gentian violet for 1 to 2 hr. and examining without additional treatment. When mounts of 2 per cent agar were prepared by the paraffin

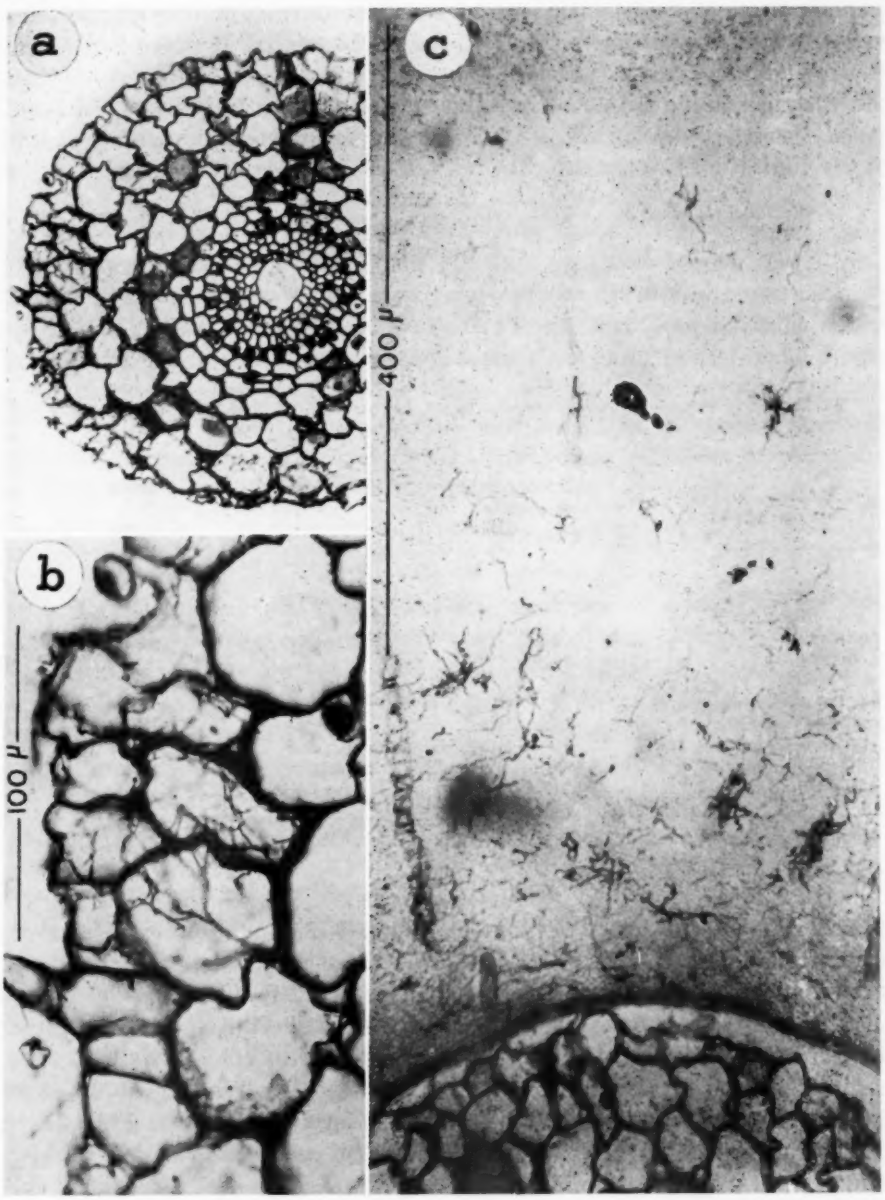

FIG. 4. Transections of wheat root grown 10 days in soil agar infested with culture 7 of *Streptomyces scabies*: a (×120), b (×400), showing *S. scabies* filaments in the cortical and epidermal cells; c (×200), with surrounding agar, showing stimulation of growth of *S. scabies* in the rhizosphere.

method, stimulation of growth in the rhizosphere was readily demonstrated with both wheat and radish. Filaments of *S. scabies* were very sparse in

the medium beyond the rhizosphere. Exact measurements of the distance from a root over which stimulation of growth was apparent could not be made because of slight shrinkage of the agar during processing. In the case of wheat, the distance was measured as 0.3 mm. after processing (Fig. 4, C), and with radish it was approximately 1 mm. It is suspected that this distance might be markedly influenced by age and debility of the root. There was no evidence that nonparasitic culture 6 was stimulated to grow in the vicinity of the root of either wheat or radish.

INFLUENCE OF CULTURE FILTRATES OF STREPTOMYCES SP. ON GROWTH OF WHEAT ROOTS

Since mycelial growth of *Streptomyces scabies* was markedly stimulated in the rhizosphere of wheat and radish roots, it seemed possible that roots might have been injured by toxic substances liberated during growth of

TABLE 5.—*Growth of wheat seedlings on soil agar containing viable inoculum and filtrates of Streptomyces sp. cultures grown on potato-dextrose agar*

Material added to soil agar	Fresh root weight[a]		Secondary root development	Root tip color
	Test 1	Test 2		
	mg.	*mg.*		
Water control	139	67	extensive	white
Viable inoculum:				
culture 6[b]	147	66	extensive	white
culture 7[b]	79	30	none	orange to brown
Filtrates:				
potato-dextrose agar	.....	50	extensive	white
20-day-old culture 6	.....	63	extensive	white
20-day-old culture 7	.....	56	extensive	white
50-day-old culture 6	121	56	extensive	white
50-day-old culture 7	121	56	extensive	white

[a] Records after 16 days, Test 1, 3 beakers each containing 5 plants; Test 2, 4 beakers each containing 5 plants.
[b] Culture 7, *S. scabies;* culture 6, nonpathogenic *S.* sp.

S. scabies. In order to investigate this possibility, aqueous extracts were made from 5 potato-dextrose agar mats on which *Streptomyces* sp. had been grown at 20° to 25° C. Mats were soaked for 12 to 15 hr. in 200 ml. of distilled water and the extracts sterilized by passage through a Seitz filter. Filtrates were apparently sterile since *Streptomyces* colonies failed to develop when the filtrates were streaked on potato-dextrose agar. Twenty-five ml. of the filtrate, representing the amount from 5/6 of a Petri plate culture, was placed in each beaker and 25 ml. of cool melted soil agar added and mixed. Viable inoculum consisting of a suspension of spores and aerial hyphae represented the amount obtained from approximately 1/5 of a Petri plate culture.

Growth of wheat (Table 5) in the soil agar with viable inoculum from cultures 6 and 7 was similar to that obtained in previous tests. The soil agar plus filtrates of aqueous extracts of potato-dextrose agar supported

vigorous growth of bacteria which reduced the growth of wheat slightly. In spite of the bacterial contamination encountered, wheat plants grew well, but not so well as in the water controls. Filtrates of aqueous extracts of cultures 6 and 7 supported some bacterial growth but not to the same extent as the filtrate of potato-dextrose agar alone. Approximately equivalent root weights were produced in soil agars containing filtrates of nonparasitic culture 6 or those of parasitic culture 7, which indicated that if a toxic material capable of inhibiting plant growth was produced by *Streptomyces scabies*, it was not demonstrated.

Roots in all filtrates were white at the tips, laterals were abundant, and root hairs were plainly evident. In the agar with viable inoculum of culture 7, root tips were necrotic and root hairs were either absent or very sparse. The reason why plant growth in test 1 should be almost twice as great as that in test 2 is not understood since the tests were prepared in January and March of 1947. In spite of the differences in over-all growth between the two trials, the differences between treatments within each trial were similar. In addition to the two tests reported, one preliminary trial was conducted with similar results[6].

INFLUENCE OF INOCULUM CONCENTRATION ON GROWTH OF WHEAT SEEDLINGS

During the course of the work, growth responses of wheat seedlings to *Streptomyces scabies* were constant in spite of the fact that it was occasionally necessary to use inoculum of different concentrations and ages. In general, the inoculum from 1/4 to 1/6 of a Petri plate was added to each 100-ml. beaker. To determine if such concentrations were excessively heavy, serial dilutions were prepared and beakers poured in triplicate. To each beaker 5 germinated seeds were planted. As in previous trials, root weights representing the average of 15 plants were markedly reduced by *S. scabies*. The extent of growth reduction was relatively the same when plants were grown in either a heavier than usual inoculum load of *S. scabies* or in a much lighter inoculum load than previously employed (Table 6). Although the reason is not apparent, root growth in the first trial was slightly less with the inoculum from 1/64 Petri plate than in the higher concentrations. In the second trial, root weights with cultures 5 and 9 were relatively constant throughout the range of inoculum concentrations employed.

It was suspected that variation in the time interval between pouring the agar and planting the seed might influence considerably the concentration of viable inoculum. In most cases, seed were planted in beakers within 24 hr. after pouring the agar, although in a few instances planting was delayed for 48 hr. In such cases, no variation in the final reaction was detected. In order to evaluate the effect of delayed planting, inoculum

[6] In one other test which was at variance with those reported, some inhibition of root growth was obtained with filtrates of culure 7 and little to no inhibition with culture 6. It is suspected that bacterial growth on the filtered medium was a factor in the aberrant result.

was added and agar poured into one group of covered beakers and maintained at laboratory temperatures. Similar inoculum in Petri dishes and soil agar in flasks was held at 8° C., and after 13 days a second set of beakers was prepared. Beakers had been prepared in triplicate, and on the following day five wheat seedlings were planted to each beaker of both groups. The average fresh root weight after 13 days' growth in the delayed planting as compared to the usual planting procedure is as follows: water control, 90 and 87 mg.; culture 6 of *Streptomyces* sp., 87 and 70 mg.; culture 4 of *S. scabies*, 40 and 47 mg.; culture 5 of *S. scabies*, 24 and 44; and culture 9 of *S. scabies*, 44 and 37 mg. No well-defined trend was apparent with delay in planting, and differences were slight in the water control and in cultures 4 and 9. Root weights with nonparasitic culture 6 were somewhat lower when planting was not delayed, whereas the reverse obtained with *S. scabies* culture 5. It was concluded that the concentration of viable inoculum was influenced little, if any, by delay in planting,

TABLE 6.—*Root weight of wheat seedlings in soil agar with varying initial concentrations of inoculum*[a]

Trial number	Culture number	Fraction of Petri plate culture used as inoculum per beaker[b]									
		1/2	1/4	1/8	1/16	1/32	1/64	1/128	1/256	1/512	0
		mg.	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*
1	7	56	57	53	51	47	42	.....	.....	.....	87
1	6	85	.....	.....	.....	.....	.....	.....	.....	.....	.....
2	5	.....	27	21	21	21	18	20	21	.....	92
2	9	.....	37	34	35	27	34	30	37	35	91
2	6	.....	91	.....	.....	.....	.....	.....	.....	.....	.....

[a] Average root weight from 15 plants grown in agar 13 days.

[b] The inoculum from 1/2 Petri plate culture represents the aerial mycelium and spores developed on 32 cm.^{2} of agar surface. (1/256 Petri plate, 1/4 cm.^{2})

since with three cultures there was no appreciable effect of delayed planting, and with two cultures reverse effects were obtained.

ROOT DEVELOPMENT IN SOIL AGAR INFESTED WITH VARIOUS STREPTOMYCETES AND BACTERIA

Soybean and radish seedlings were grown in soil agar artificially infested with 24-hr. cultures of each of the following ten members of the Eubacteriales:[7] *Serratia marcescens* Bizio, *Bacillus subtilis* Cohn emend. Prazmowski, *Bacillus mesentericus* Trevisan, *Escherichia coli* (Migula) Castellani and Chalmers, *Micrococcus pyogenes* var. *aureus* (Rosenbach) Zopf., *Sarcina subflava* Ravenel, *Aerobacter aerogenes* (Kruse) Beijerinck, *Proteus* sp., *Pseudomonas ovalis* Chester, and *Xanthomonas phaseoli* var. *sojensis* (Hedges) Starr and Burkholder. In addition, three unidentified bacterial cultures which had developed as contaminants on the soil agar in

[7] Dr. S. T. Chao of the Botany Department supplied the culture of *Xanthomonas phaseoli* var. *sojensis* and Dr. Noel H. Gross, formerly of the Department of Bacteriology, provided the other cultures tested.

previous trials were tested. Although the older portions of the roots were discolored by some of the cultures, *X. phaseoli* var. *sojensis* was the only culture which caused discoloration of the root tips of soybean and radish. The reaction was distinct from that obtained with cultures of *Streptomyces scabies* prepared at the same time, indicating that the response to *S. scabies* was specific and not obtained with any of the 13 cultures.

Ten *Streptomyces* sp. cultures isolated on soil agar from corn land that had not recently been cropped to potatoes were tested on soybean and radish[8]. None of these cultures produced a reaction typical of that already described for these plants, whereas cultures of *S. scabies* prepared under similar conditions produced a typical response.

A group of 24 *Streptomyces* sp.[9] was tested on soybean. At the time Taylor and Decker tested these cultures, 16 were nonpathogenic to potato, four were very virulent, and four were slightly virulent to potato. After approximately 10 years in culture (22), three of the four very virulent cultures were still virulent when tested on soybean, one of the four slightly virulent cultures had maintained its virulence, and none of the 16 nonpathogenic cultures was virulent on soybean. One representative culture, number 407, pathogenic to soybean and wheat, was tested on potato and found to be extremely virulent.

Ten *Streptomyces* sp. isolated from soil pox lesions on sweet potato roots were tested twice on soybean and once on wheat. Of the group, only one was typically parasitic. When this culture was used to infest peat soil in which potatoes were planted, typically scabbed tubers were obtained. It is probable that this organism was *S. scabies* rather than *S. ipomoea* since it was not pathogenic to sweet potato in greenhouse tests.

Two cultures of the sweet potato soil pox organism, *Streptomyces ipomoea* (Pearson and Martin) Waksman and Henrici[10], were tested twice on wheat and slight root tip necrosis was obtained. The reaction, although not so severe as that obtained with *S. scabies*, was very similar. These cultures did not cause scab on Cobbler potatoes in greenhouse tests.

Streptomyces albus (Rossi, Doria, emend. Krainsky) Waksman and Henrici and *S. antibioticus* (Waksman and Woodruf) Waksman and Henrici[11] were tested on wheat and pea with no reaction typical of that caused by *S. scabies*.

Streptomycin produced by *Streptomyces griseus* (Krainsky) Waksman and Henrici has been reported (1) to stunt the roots of various plants such as tomato, radish, and soybean. When cultures of *S. griseus* were used on wheat, radish, beet, and soybean, roots, particularly the laterals, were stunted with no visible necrosis. The reaction was distinct from that ob-

[8] C. H. Meredith, formerly of this department, provided these cultures.

[9] The author is indebted to Carlton F. Taylor and Phares Decker for the use of these cultures, and to Wilson L. Smith, Jr., of Cornell University, who sent the transfers.

[10] One culture was isolated from soil pox infected sweet potatoes in Iowa, and a second culture was provided by Dr. W. J. Martin of the Louisiana Agricultural Experiment Station.

[11] Dr. S. A. Waksman of the New Jersey Agricultural Experiment Station made these cultures available.

tained with *S. scabies* although over-all root growth was markedly reduced. Cultures of *Streptomyces* sp. were subsequently isolated from Iowa peat soils which were similar in their effect on wheat. With these soil isolates, roots were markedly stunted without macroscopically visible necrosis. Representative cultures causing this reaction on wheat were nonpathogenic to potato in greenhouse inoculation tests.

Streptomyces scabies cultures 4 and 9, isolated in January, 1944, and cultures 5 and 7, isolated in August of the same year, were transferred frequently on potato-dextrose agar. Most of this experimental work was done between January, 1946, and March, 1947. After 30 months, culture 7 lost its ability to parasitize seedling plants. The loss of pathogenicity was correlated with loss of ability to cause scab on potato in greenhouse inoculation tests. This was interpreted as further evidence that there was a positive correlation between parasitism to potato and ability to cause root necrosis and reduction in secondary roots of test plants. The pathogenicity of cultures 4, 5, and 9 remained constant over the period of observation.

DISCUSSION

The present studies show that *Streptomyces scabies* attacks a wide range of host plants under favorable conditions. The plants studied are unable to survive in artificially infested peat soil. Root infection occurs in quartz sand, and extensive root necrosis is consistently observed in soil agar. Because of the following facts the data should not be dismissed as indicating saprophytic development of *S. scabies* on weakened plants: (1) *Streptomyces* sp. nonparasitic to potato failed to attack roots of wheat, soybean, and pea; (2) the true bacteria that were tested failed to attack roots of such plants in a manner similar to that of *S. scabies;* and (3) differences in resistance to *S. scabies* were observed in the plant species tested.

Symptoms on roots grown in soil agar consisted of necrosis of root tips with a marked reduction in the number of lateral roots. Lateral roots of infected bean and pea were short stubs, whereas they generally failed to develop in infected wheat. Punctate necrotic areas on the roots of wheat were points of emergence of lateral roots in which the developing tip had been severely attacked by *Streptomyces scabies.* More resistant plants, such as certain cucurbits and corn, produced lateral roots that were shortened and noticeably thickened. Similar symptoms have been described on potato. Millard and Burr (14), using artificially infested soil, observed that the fibrous roots of potato were stunted and somewhat thickened and thickly studded with irregular dark brown outgrowths. Jones (8) demonstrated similar outgrowths to be lateral rootlets which had been partially destroyed by *S. scabies* as the lateral root emerged from the main root.

Observations on prepared sections showed that mycelium of parasitic cultures of *Streptomyces scabies* developed in the epidermal and cortical cells, becoming well established before macroscopically visible necrosis developed, whereas under similar conditions no penetration of root tissue was observed with a nonparasitic species.

In the field, streptomycetes, as well as other types of soil micro-organisms, have been reported to be much more abundant in the rhizosphere than in the soil away from the roots (9, 10, 21). In agar culture, *Streptomyces scabies* developed abundantly in the rhizosphere, whereas a nonparasitic species was not stimulated to grow in the rhizosphere. It was not determined whether *S. scabies* was stimulated to grow in the rhizosphere before actual penetration because of excretions from the healthy root, or whether *S. scabies* was stimulated to grow in the rhizosphere after penetration because of nutrients diffused from the parasitized root.

Culture filtrates of *Streptomyces scabies*, when tested under a limited set of conditions, apparently were no more toxic to wheat plants than were filtrates of a nonparasitic streptomycete. Inoculum dilution tests showed that *S. scabies* was equally effective in soil agar over a wide range of initial inoculum concentrations. Although it had been shown that growth of *S. scabies* was very poor on soil agar, it is possible that the uniform disease reaction obtained over a wide range of initial inoculum concentrations may have resulted either from growth in the low initial concentrations or from incomplete survival of inoculum in the high concentrations. These results are in contrast to those of Goss (4) in which the severity of potato scab in sterilized artificially infested soil was directly correlated with initial inoculum concentration.

No claim is made that these tests duplicate natural conditions. They do demonstrate that *Streptomyces scabies* is innately virulent to the roots of a number of plants not previously believed to be suscepts of *S. scabies*. It does not necessarily follow that the disease will be produced in the field even though it is suspected of being of more consequence on some crops than is ordinarily believed. It seems entirely possible that *S. scabies* could compete successfully with other organisms and parasitize plant roots. Beijerinck (2) reported streptomycetous filaments of *Streptothrix chromogena* Gasperini in the roots of various plants grown in the field. This organism may or may not have been *S. scabies;* his identification was based chiefly on cultural characteristics.

The presence of soil saprophytes influences considerably the parasitic ability of *Streptomyces scabies* in infested soil although published data are not in complete agreement in many details. Millard and Taylor (15) showed that in sterilized soil infested with *S. scabies*, considerable reduction in the severity of scab was obtained by introducing *S. praecox* as an antagonist. Kiessling (12) reported similar results with bacteria. Although Goss (4) was unable to obtain control of scab with *S. praecox*, he showed that scab was much more severe in sterilized, artificially infested soil than in similarly infested soil to which soil saprophytes had been introduced. Sanford (17) obtained more severe scab injury in the field by placing unsterilized, heavily infested soil around the seed piece, than by using sterilized, infested soil. KenKnight (11) experienced considerable difficulty in determining the host range of *S. scabies* because of contamina-

tion of controls by *S. scabies*. Others (22, 23), in greenhouse inoculation experiments with potato, have reported no infection or only rare infection in their noninoculated controls. Unless the host can be maintained in a culture medium relatively free from contaminants, the final result in an inoculation experiment may be unsatisfactory. This difficulty is believed to have been partially overcome, at least for small plants, by the use of the soil-agar technique.

The use of soil agar as a medium for plant root growth, in combination with streptomycetes, makes possible the testing of cultures with considerable freedom from outside contamination since the growth of most microorganisms on this medium is sparse. The medium permitted daily detailed examination of the roots. In general, plant growth was very satisfactory and roots developed extensively. Root hairs of wheat were plainly evident. The agar was easily washed from the roots permitting quantitative measurements. A group of cultures could be screened, for those parasitic to a given host, with relative ease. Satisfactory readings on potato generally require 60 days of greenhouse culture, whereas a screening on wheat and soybean can be made in 14 days, with preliminary indications available after 7 days.

The development of a dark brown ring in separated milk culture has been reported to be characteristic of *Streptomyces scabies* (22), whereas cultures which did not develop such a ring were nonpathogenic to potato. The ten cultures described in this paper were grown on milk with fair correlation between color changes in the milk and pathogenicity to potato. No culture parasitic to potato failed to give a positive reaction on skim milk. A few exceptions were observed (Table 1) in which nonpathogenic *S. lavendulae* and *Streptomyces* sp. culture 8 produced a color in milk which was indistinguishable from that of certain *S. scabies* cultures.

Although the strong possibility exists that certain isolates of *Streptomyces scabies* may be virulent to potato and avirulent to seedling test plants, it was found that a high correlation existed between pathogenicity to potato and pathogenicity to seedling plants when grown with *S. scabies* under the conditions employed. *S. ipomoea* isolates parasitic to sweet potato and nonparasitic to white potato were mildly pathogenic to wheat. Physiologic races were not demonstrated with the four *S. scabies* and six *Streptomyces* sp. cultures on such widely divergent plant hosts as soybean, pea, wheat, corn, beet, radish, and cucumber, although physiologic races as demonstrated on potato varieties and variability in *S. scabies* have been reported (19, 23).

SUMMARY

The host range of *Streptomyces scabies* on seedling plants was studied by using sterilized artificially infested peat soil, infested quartz sand, and soil agar. Scab infection of seedling plants grown in artificially infested peat soil in open pots in the greenhouse was generally unsatisfactory because of contamination. Infection of plants grown in infested peat in large

plugged test tubes was severe, resulting in infection of roots and aerial parts and premature death. *S. scabies* parasitized wheat and soybean roots developing in quartz sand, although necrosis was neither so severe nor so consistent as that on roots in soil agar. A soil-agar culture technique proved useful because cultures could be maintained relatively free from contaminants. Chronological examination of roots was possible and clean roots free of mechanical injury could be recovered.

In soil agar, four cultures of *Streptomyces scabies* caused severe necrosis, especially at the root tips, and generally precluded the development of lateral roots on soybean, pea, wheat, radish, and beet, whereas corn had slight tolerance, and cucumber considerable tolerance. Isolates of *Streptomyces* sp. that were avirulent to potatoes did not adversely affect such plants. In addition to the plants listed above, susceptible reactions to *S. scabies* were obtained with *Avena* spp., *Hordeum* spp., *Triticum* spp., rye, eggplant, tomato, carrot, parsnip, and potato seedlings. Cucurbits generally had a high degree of tolerance.

Virulent *Streptomyces scabies* cultures invaded the epidermal and cortical cells of wheat and radish roots and became intracellular. Filaments were much more abundant in the rhizosphere than in the medium a short distance away. One avirulent strain was not evident intracellularly nor was it stimulated to grow in the vicinity of the root. Aqueous extracts of *S. scabies* cultures were no more toxic to wheat roots than extracts of cultures nonparasitic to potato. *S. scabies* was equally parasitic to wheat roots over a wide range of initial inoculum concentrations, and the growth response was constant where planting was delayed by as much as eight days.

Although a total of 60 *Streptomyces* sp. cultures were tested on wheat, with the exception of *S. ipomoea*, only those known to cause potato scab parasitized wheat seedlings. *S. ipomoea* caused necrosis of wheat roots in a manner similar to that of *S. scabies*, although the reaction was much less severe. Root necrosis was not obtained when cultures of 13 members of the Eubacteriales were tested on seedlings growing in agar. In the few instances where root discoloration developed, the reaction was distinct from that caused by *S. scabies*.

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A LATE-BREAKING VIRUS DISEASE OF POTATOES[1]

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In 1946 an unusual disease of potatoes became prominent in many potato fields in Oregon, most fields of Netted Gems, Burbanks, and White Rose having from a trace to 3 per cent of the disease. After all seed lots with more than 1 per cent infection were rejected for certification, prevalence was lower in 1947 plantings. Seed obtained directly from Montana, Colorado, and Idaho produced plants with tuber-perpetuated symptoms, an indication that the disease is rather widespread.

Vine symptoms suggested Rhizoctonia and leafroll complexes, but closer observations indicated that neither disease was involved. Subsequent studies demonstrated the disease to be of virus nature, but possessing certain features which indicated that it was different from viroses heretofore associated with potatoes in Oregon. The extensive distribution of the disease in Oregon and its unusual nature suggested this preliminary paper.

SYMPTOMS

This disease was first noted in the Netted Gem variety, in plants which appeared to be showing the effect of current season infection. Most of the affected plants were of normal size and frequently only one branch or even one lateral was affected. However, when studied in a field planted in tuber units it became apparent that the disease was tuber-perpetuated.

When the symptoms are first expressed, the tips of the laterals begin to roll much as they do when the roots are injured by Rhizoctonia or some other factor, or when the plants are infected with current season leafroll. The rolling closely resembles that illustrated by Leach and Bishop (7) for Purple-top wilt. Subsequent symptom development is from the tip downward until the entire plant becomes involved. In a few days the plants become stiff and erect, with olive green to chlorotic foliage. They often have a purplish cast from heavy pigmentation of the leaf veins, the stems, and the small rolled leaflets in the branch terminals. In the more advanced stages some plants wilt and die, apparently from root starvation and subsequent rot. Most plants in the advanced stages of the disease produce aerial tubers, often in nearly every leaf axil.

The disease was studied in fields which had been planted in tuber units, and considerable variation was noted in the degree of severity of symptoms. Occasionally the entire unit was dwarfed and rolled much in the same manner as for leafroll, but for the most part the units contained

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[2] Plant Pathologist and Associate Plant Pathologist, respectively. Dr. English is now on the staff of the California Agricultural Experiment Station.

plants which had developed to nearly normal size before expressing symptoms. In some units all plants were affected in a uniform manner, but in others normal plants, plants with mild symptoms, and plants with acute symptoms could be found, all from the same tuber.

Occasionally a seed piece may not produce a plant large enough to reach the surface of the soil. Short stolons with small terminal tubers are the only structures produced (Fig. 1, C). The seed pieces from diseased

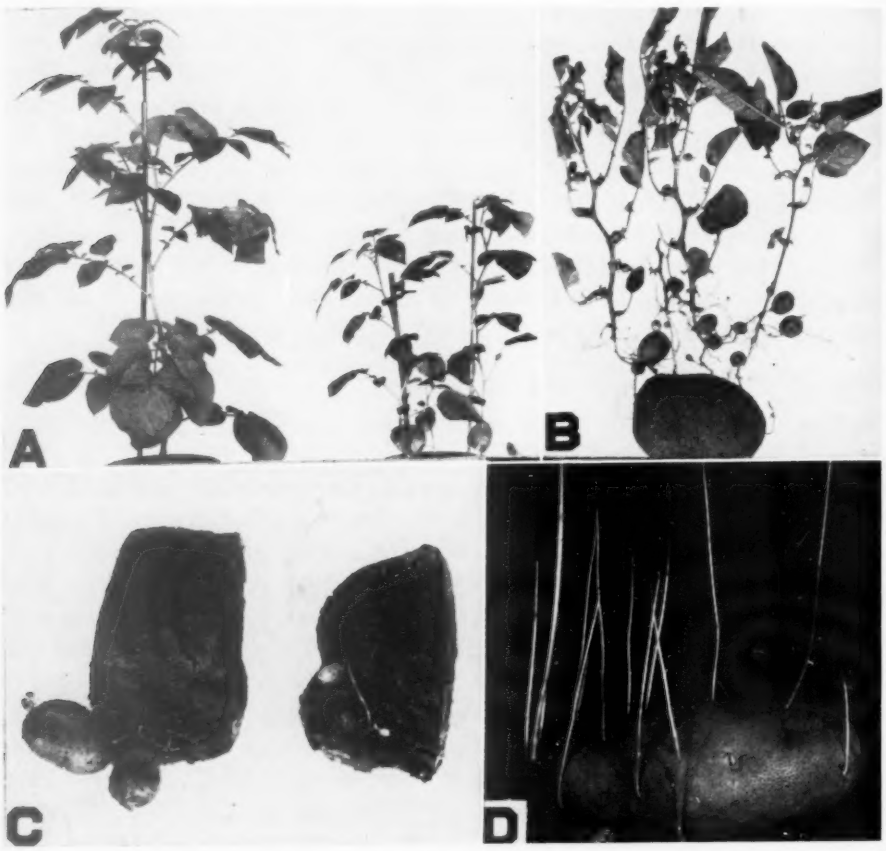

FIG. 1. Late-breaking virus disease of Netted Gem potato. A. Right, weak plant from infected tuber grown in greenhouse; left, a normal plant. B. Field plant with early acute symptoms. C. Dormant seed piece with small secondary tubers. D. Infected Netted Gem tuber producing small weak sprouts.

tubers are resistant to breakdown and may remain firm in the soil for several months. Some seed pieces produce knots along the cut vascular ring, while others force out a well defined root system from the cut vascular ring.

In general, the appearance and behavior of the disease in the White Rose and Burbank varieties is similar to that in Netted Gems. However, the late-breaking or late-developing symptoms are not so striking on White

Rose as on Netted Gems, and the disease is extremely difficult to distinguish by foliage symptoms from that caused by Rhizoctonia or other similar root injuries. Its expression usually is limited to an apical leafroll followed by aerial tuber development in nearly every leaf axil. If a White Rose plant shows the foliage symptoms described above and there

FIG. 2. Tuber-perpetuated late-breaking virus disease of White Rose potato. A. Aerial tubers. B. Downcurving axillary branches. C. Plant terminal 60 days after inoculation by grafting. D. Aerial stolons.

are no evident root injuries and the tubers are smooth and small, the disease will be reproduced by many of the tubers. Some infected White Rose plants develop the early and acute symptoms that resemble tuber-perpetuated leafroll. The plants are dwarfed and chlorotic as for leaf-

roll but they do not express the rolling, cupping, and bronzing of the lower leaves.

Tubers from acutely diseased plants are reduced in size but have no internal discoloration or other evidence of disease. Most of the tubers are firm and show no indication of wilting or flabbiness unless the plant suddenly wilts and dies from what appears to be a secondary invasion of the weakened or starved roots by Fusarium and other soil organisms. Many of the larger tubers from infected hills produce hair-sprouts, either from all of the eyes or from a portion of the tuber (Fig. 1, D).

Tubers taken from infected hills of Netted Gems and planted in the greenhouse produced plants varying greatly in symptom expression.

TABLE 1.—*Location of the virus in the tuber*

Tuber number	Apical left	Basal left	Apical right	Basal right
1–30	–	–	–	–
31	–	+	–	+
32	+	+	+	+
33	+	+	–	+
34	–	+	+	+
35	–	+	–	–
36	+	–	+	–
37	+	–	+	–
38	+	–	+	–
39	–	+	–	+
40	+	+	+	+
41	+	+	+	+
42	–	+	–	+
43	–	–	–	+
44	–	+	+	+
45	+	–	+	–
46	–	+	+	–
47	–	+	–	+
48	+	–	+	–
49	+	+	+	+
50	–	+	+	+

Often the plants appear normal or vary only slightly from normal. There is a decided tendency for delayed emergence, and for weak (Fig. 1, A) and hair-sprout plants. Occasionally a plant will develop in a normal manner until 17 in. or more tall, then terminal growth will cease and purple aerial tubers or short, vase-shaped shoots will develop in the axils of the leaves as illustrated for White Rose in figure 2. These plants soon become chlorotic and when they reach a certain stage of development many of them wilt and die within a few days.

Symptoms in greenhouse-grown White Rose are much more clear-cut than on Netted Gems. In addition to dormant seed pieces, hair sprouts, and weak plants, nearly every infected plant may produce profuse axillary aerial tubers, abnormal, downward-curving leafy branches, or aerial stolons with or without tubers attached (Fig. 2, A, B, D).

In potato fields, several plants were noted with two stalks from the

same seed piece: one stalk had acute symptoms and tubers were small; the other appeared to be healthy, with normal tubers. In an experiment, one tuber from a diseased stalk and another from a normal stalk, taken from each of ten different hills, were segregated and planted in the greenhouse. All of the tubers from the diseased stalks produced diseased plants; those from the normal stalks produced nine normal plants and one diseased plant.

Fifty tubers weighing from 6 to 8 ounces were selected from hills having mild symptoms of the disease. Each tuber was cut once longitudinally and once crosswise, and the four seed pieces were placed in field plot rows so that the pieces were oriented as to their position in the tuber. Table 1 shows the results of these studies.

Thirty of the tubers produced apparently normal plants from all four seed pieces. The other 20 tubers produced from 1 to 4 diseased plants. The virus was not always present throughout the tuber, but was often localized in some one area.

INOCULATIONS

Netted Gems and White Rose have been inoculated by grafting, using infected plants of both varieties as sources of the disease. Approximately 60 days after grafting a portion of the infected plant onto small healthy plants, an apical leafroll developed and axillary tubers appeared in the upper portions of the plants (Fig. 2, C). Inoculated plants became decidedly yellowed and the leaves rolled upward. Plants grown from tubers produced by the inoculated Netted Gem plants, whether or not current season symptoms were evident, invariably developed typical symptoms. The progeny from the inoculated White Rose plants, however, failed to produce symptoms, again regardless of current season expression of the disease. Check plants originating from the same tubers as the inoculated plants remained normal throughout the experiment.

Although the disease is of the yellows type which is usually not transmitted by juice inoculations, five plants were inoculated using carborundum powder and the juice taken from plants having early but well-defined symptoms of the disease. The plants were grown to maturity but none developed any type of abnormal growth that differed from the uninoculated check plants. Table 2 summarizes the results of the inoculation studies.

DISCUSSION

The name "late-breaking virus disease" has been proposed and is being used locally as the common name of the disorder. The name is derived from the fact that the disease, even when tuber-perpetuated, often does not express itself until late in the season at which time its development is very rapid.

For a comparison with other similar virus diseases or disease complexes

described for potatoes, the following points pertinent to the late-breaking virosis should be noted: (1) repeated demonstrations of tuber perpetuation for two or more generations; (2) no internal necrosis of stems or tubers; (3) tubers usually borne on short stolons, small but not in chains or excessive in number; (4) profuse production of aerial tubers in the field and aerial tubers and aerial stolons in the greenhouse; (5) uneven distribution of the virus in the tuber; (6) hair sprouts from large tubers but no tendency for multiple sprouting or branching to produce witch's-broom effect; (7) the production of dwarf plants that show acute symptoms early (Fig. 1, B), or apparently normal plants that develop symptoms late in the season, often from the same tuber.

This disease has many features which suggest that it belongs to a group of viroses which for the most part have not been sufficiently investigated to clearly demonstrate their relationships. Its erratic behavior and dif-

TABLE 2.—*Inoculation studies demonstrating the transmission of late-breaking virus disease to White Rose and Netted Gem potatoes*

Variety	Source of inoculum	Type of inoculation	Number of plants	No. of transmissions	
				Current season	Tuber-perpetuated
White Rose	White Rose	graft	9	7	0
White Rose	Gems	graft	6	5	0
Gems	Gems	graft	11	4	11
Gems	White Rose	graft	9	7	9
White Rose	Checks	none	3	0	0
Gems	Checks	none	6	0	0
Gems	White Rose	juice	5	0	0
Gems	Checks	juice	3	0	0

ferent symptom expression at times suggest relationship to Purple-top wilt (7), Yellow Top (3, 4, 9), Witch's Broom (6), Apical Leafroll (1, 8), Haywire (5), and Bunch top (2). However, the points listed above appear to differentiate this "late-breaking" virus disease from these previously described and somewhat similar disorders.

This disease does not have the constant, clear-cut symptom expression of many of the other potato virus maladies such as mosaic and leafroll. This will make it difficult for growers to eliminate it from their seed stocks. The delayed symptom development and the uneven occurrence within a tuber makes the disease hard to detect in eye index and winter test plots. The tendency for the majority of infected Netted Gem tubers to develop only weak or leggy plants with no definite foliage symptoms makes greenhouse detection and study difficult. For the same reason trouble is being experienced in vector studies that now are in progress. This virus disease presents a number of intriguing research problems that remain to be solved.

SUMMARY

An unusual virus disease has been found in Oregon which possesses certain characteristics that differentiate it from other viroses associated with potatoes. It has been called "late-breaking virus disease" because the symptoms on plants produced from infected tubers usually develop late in the season. The disease has been tuber-perpetuated for at least two generations and has been transmitted to healthy plants by grafting. The disease is very erratic in behavior and symptom expression, a fact that will make it difficult to detect and eradicate from seed stocks.

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A CRATER SPOT OF CELERY PETIOLES CAUSED BY RHIZOCTONIA SOLANI

BYRON R. HOUSTON AND J. B. KENDRICK[1]

(Accepted for publication February 20, 1949)

For many years a petiole crater spot of celery has been present on plants in the fields in the Sacramento-San Joaquin Delta of California. The severity of the disease varies to some extent with the season and usually increases with continuous celery culture. Losses result when the outer petioles become infected and must be removed before marketing. Often the infections occur to such an extent that excessive trimming makes it impossible to market the small or improperly shaped plants. Both the Pascal and Self Blanching types of celery are susceptible. The investigations herein reported were undertaken to determine the primary cause of this disease.

The first evidence of the disease is seen on the outer petioles in contact with the soil. Infections occur on either the dorsal or ventral surface of the petiole. The lesions first appear as small tan spots (Fig. 1, B, C) which may show a slight margin of watersoaked tissue if in very moist soil. As the spots enlarge they become darker brown and sunken in the center as the tissues collapse and dry (Fig. 1). On the dorsal surface the lesions are often elongated an inch or more in the direction of the long axis of the petioles. The parenchyma tissue between the veins collapses leaving the veins as ridges through the spots (Fig. 1, B). Infections several inches above the crown occur after ridging of the soil against the plant a short time prior to harvest to produce a partial blanching. Infections occurring on rapidly elongating petioles result in a scarring of the surface tissue and often a distortion (Fig. 1, F).

Isolations made in the fall of 1947 from field specimens of the disease yielded a number of fungi and bacteria. The most common fungi isolated were *Rhizoctonia solani* Kuhn and what appeared to be *Fusarium solani* (Mart.) App. et Wr. emend. Snyder and Hansen. *Erwinia carotovora* was isolated many times, as were a number of other bacteria. Ark (1) reported a crater rot of celery from the Delta region as being caused by *E. carotovora*. His published photographs of the disease show it to be very similar to the disease reported here. Bardin and Fong (2) reported a Phoma root and crown rot of celery from California which produces dark-brown to black lesions around the base of the petioles and on the roots, but does not produce sunken spots with a sharply limited margin. This was apparently the same disease as that earlier described by Bennett (3). In 1907 Van Hook (5) reported a celery root rot from Ohio with which *Rhizoctonia* was

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associated, but he was unable to reproduce the disease in inoculations with this organism.

The pathogenicity of the various fungi and bacteria isolated from the

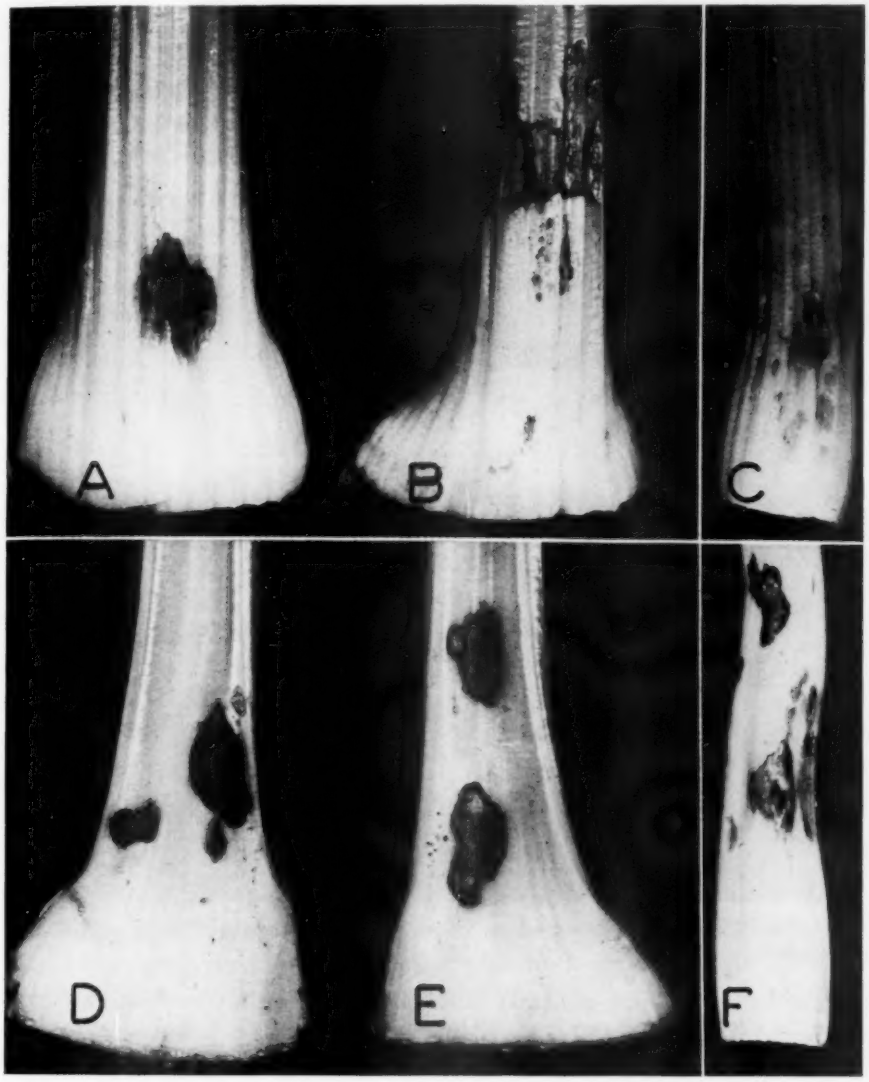

FIG. 1. Field specimens of celery petiole rot caused by *Rhizoctonia solani*. A, B, and C. Dorsal surface of petiole. D, E, and F. Ventral surface of petiole. A, D, and E. Typical sunken lesions following the drying of the affected tissue. B. Elongate lesion on dorsal surface showing sunken tissue between veins. C. Young infections somewhat watersoaked prior to discoloration. F. Multiple infection of young petiole resulting in distortion.

field specimens was tested in the greenhouse in the spring of 1948. Celery plants of the Pascal type approximately 4 months of age growing in deep flats of sterile peat soil were inoculated by pouring macerated agar cultures

of the organisms around the crown of the plant and between the petioles. The soil was then pulled up around the base of the petioles and kept moist for 3 weeks. Ten plants were inoculated with each organism. Preliminary observations at the end of 5 days showed that none of the organisms had penetrated the tissues of the celery petioles, with the exception of *Rhizoctonia solani* which had produced small tan lesions 4 to 8 mm. in diameter on both the dorsal and ventral sides of the petioles. At the end of the 3-week period the plants were dug and examined and it was found that no organisms other than *R. solani* had infected the petioles. This

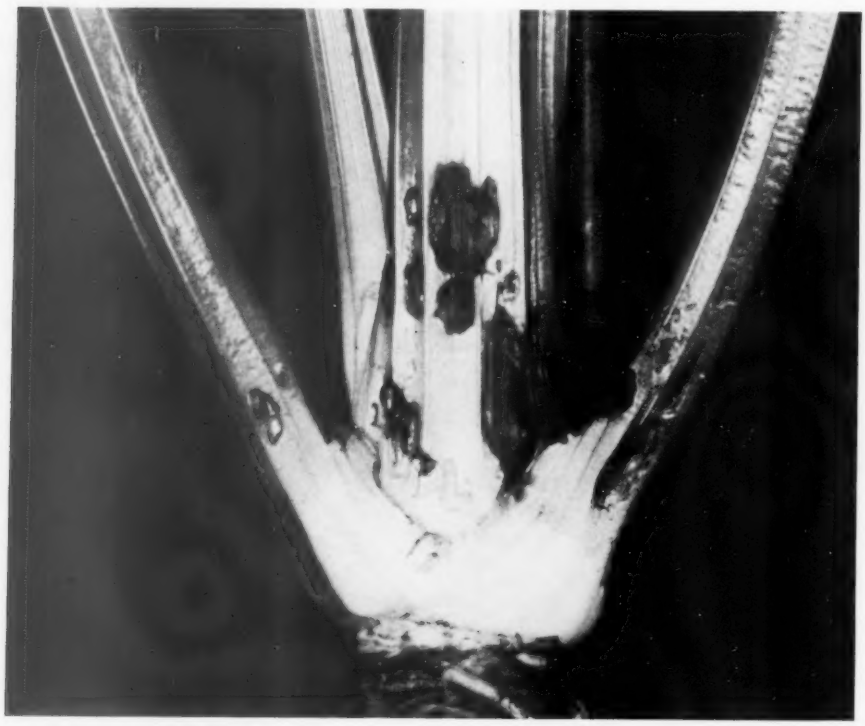

FIG. 2. Lesions on celery petioles following greenhouse inoculations with *Rhizoctonia solani*.

fungus had produced multiple infections with resulting lesions several centimeters in width (Fig. 2). Isolations from these infections were approximately 90 per cent *R. solani* which appeared identical to those isolates used for inoculation.

It has been found difficult to consistently isolate *Rhizoctonia solani* either from field specimens or greenhouse inoculations because of secondary bacterial contaminants of nonpathogenic types which tend to suppress the growth of the *Rhizoctonia*. Good results were obtained by isolating from the younger infections with the use of acidified potato-dextrose agar.

Inoculations with isolates of *Rhizoctonia solani* obtained from sugar beet

and potato stem lesions showed these isolates to be very pathogenic on celery. All of the isolates obtained from natural celery infections as well as those from other hosts were found to be of the type designated as type A by Houston (4).

The results of soil inoculations are given in table 1. Four isolates of *Rhizoctonia solani* and one of *Erwinia carotovora* from celery were mixed into flats of soil prior to planting with healthy celery plants about 3 months old. In these trials *R. solani* caused the death of about 17 per cent of the transplants by complete invasion of the crown tissues subsequent to the crater spot. The remainder of the plants were all severely damaged by typical crater-spot petiole infections. Infection by *E. carotovora* occurred at a few places on the outer petioles apparently at points of injury.

Further trials with *Erwinia carotovora* on growing plants and on excised petioles in moist chambers showed that this organism was capable of entering the tissue only when a wound was present. Puncturing with a

TABLE 1.—*Crater spot infection following the infestation of sterile soil with cultures of Rhizoctonia solani and Erwinia carotovora isolated from celery prior to planting with healthy celery plants*

Isolate number	Number of plants:		Average severity of crater spot infection[a]
	Planted	Surviving	
R. solani 1	40	32	2.6
R. solani 2	40	39	2.9
R. solani 4	40	35	3.0
R. solani 7	40	27	2.6
E. carotovora + 1	40	40	0.2
Sterile soil	40	40	0.0

[a] Rating based upon number of crater spot lesions on petioles and number of petioles infected: 0 = healthy and 4 = dead.

needle at the time of inoculation resulted in infection and the formation of a typical crater of watersoaked tissue followed by collapse of the internal parenchyma of the petiole several centimeters in each direction from the point of inoculation. No typical crater spot was produced. When a suspension of *E. carotovora* was poured around the crown of plants which had 5 days previously been inoculated with *Rhizoctonia solani* the bacteria became established in the areas injured by the fungus on 12 of the 50 plants inoculated. On 7 of the 12 plants the bacteria completely invaded the crown tissues resulting in the death of the plant. A soft rot occurred on some of the petioles of the other 5 plants.

The evidence presented indicates that *Rhizoctonia solani* is the primary cause of crater spot and that *Erwinia carotovora* will produce a soft-rot lesion by invasion through mechanical wounds or through lesions produced by *R. solani*.

SUMMARY

A petiole crater-spot of celery was found rather widely distributed in the Delta region of California. The disease was characterized by tan to

brown, ovoid, sharply sunken lesions on the dorsal or ventral surface of the petiole often somewhat more elongated on the dorsal surface.

Although a number of fungi and bacteria were isolated from the diseased plants, the only organism which proved to be pathogenic was *Rhizoctonia solani*. Isolates of this fungus from sugar beet and potato stem lesions also were pathogenic on celery.

Erwinia carotovora produced petiole rot when inoculated through mechanical wounds on celery petioles or when inoculated subsequent to *R. solani* infections. In the latter case complete invasion of the crown tissue may occur.

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DECAY OF CERTAIN NORTHERN HARDWOODS BY FOMES IGNIARIUS, PORIA OBLIQUA, AND POLYPORUS GLOMERATUS

RAY R. HIRT

(Accepted for publication February 24, 1949)

One of the most important heartrot fungi of nonconiferous trees, *Fomes igniarius* (L. ex Fries) Gill., is common in hardwood stands of New York State. At the Charles Lathrop Pack Demonstration Forest at Warrensburg, New York, it occurs frequently on hardwood trees of various species and is especially common on aspen. The sporophores of *Fomes igniarius* on aspen differ somewhat in appearance from those produced by the fungus on trees of other species. In culture, the isolates from aspen also differ in certain respects from isolates of the fungus taken from other hardwood hosts (2, 5). It has been stated that the aspen form grows only on aspen (5). If *Fomes igniarius* from aspen should be able to grow on other hardwood species, then a heavily infected aspen stand in close proximity to other hardwoods could be a serious source of inoculum and of concern in the management of hardwood stands.

In 1938 a project was begun at the Pack Forest to discover whether isolates of *Fomes igniarius* from trees of various hardwood species could grow in hardwood hosts of species other than those from which the isolates were obtained. Since sterile conks on birch and beech at that time were thought to be formed by *Fomes igniarius*, they were included in the study. It was known that sterile conks on beech were not always associated with that fungus (4).

THE ISOLATES

Isolates were taken in the spring of 1938 from the following trees bearing fertile sporophores of *Fomes igniarius*[1]: aspen (*Populus tremuloides* Michx.); beech (*Fagus grandifolia* Ehrh.); elm (*Ulmus americana* L.); and ironwood (*Ostrya virginiana* [Mill.] K. Koch). Isolates were secured also from sterile rimose conks on yellow birch (*Betula lutea* Michx.) and beech. The isolated mycelia were grown in culture and their growth characteristics compared with those recorded by Campbell (2) and by Verrall (6) for *Fomes igniarius*.

The isolates from fertile sporophores of *Fomes igniarius* and the associated decayed wood grew, with minor variations, in the characteristic manner in culture. The isolate from the fertile sporophore on beech grew more rapidly than any of the other isolates on malt extract agar. However, in all other respects it was typical of *F. igniarius* cultures described by Campbell. The isolate from aspen was similar also to Campbell's descrip-

[1] Under the direction of the author the fungi were isolated and studied in culture by the following men who were seniors in The New York State College of Forestry: Valentine Caroline, Lynn Harrington, Ira Levine, Alois W. Otto, Charles Speers, and John Woodruff. Mr. Caroline's work was done in the spring of 1939.

tion of *F. igniarius* in culture except that it had a pronounced wintergreen odor.

The fungi isolated from the sterile conks were different from *Fomes igniarius* in their cultural characteristics. The isolate from the sterile conk on beech proved to be *Polyporus glomeratus* Peck. The fungus cultured from the sterile conk on birch was identified by Ross Davidson of the Division of Forest Pathology, Beltsville, Maryland, in May, 1938, as belonging to the *Poria obliqua* complex. Fertile sporophores produced on dead trunks of yellow birch bearing sterile conks in the stand where the isolate was secured were identified by Dr. J. L. Lowe of The New York State College of Forestry as *P. obliqua* (Pers.) Bres.

INOCULATION OF HARDWOODS

Healthy, vigorously growing forest trees of trembling aspen, beech, yellow birch, sugar maple (*Acer saccharum* Marsh), and ironwood growing

TABLE 1.—*The hosts from which the three heartrot fungi were isolated and the hosts in which they became established following artificial inoculation*

Isolate and source	Success[a] of inoculation				
	Aspen	Ironwood	Maple	Beech	Birch
Fomes igniarius					
Aspen	5/5	1/1	0/1	1/1	1/1
Ironwood	1/1	5/5	1/1	1/1	1/1
Elm	2/2	1/1	3/5	1/1	1/1
Beech	1/1	1/1	1/1	4/4	1/1
Poria obliqua					
Birch	1/1	0/1	1/1	1/1	3/3
Polyporus glomeratus					
Beech	.....	.....	.....	2/2	1/1

[a] The denominator indicates the number of inoculations; the numerator, the number of successful inoculations where the fungus became established and caused decay.

in the Pack Forest at Warrensburg were inoculated with the isolates of *Fomes igniarius* and *Poria obliqua* in July, 1938. Beech and yellow birch only were inoculated with *Polyporus glomeratus* in July, 1939.

Inoculation of the trees was accomplished by drilling holes into the heartwood of the trees and inserting the agar substratum covered with vigorously growing mycelium. Care was taken to avoid contamination of the inocula during the procedure. Each hole was plugged with a sterilized birch dowel, which was immediately brushed with white lead paint. The trees were observed several times each year until they were felled in October, 1948, and split longitudinally, and the decay was studied.

RESULTS

Regardless of the host from which cultures of *Fomes igniarius* had been secured, except for the aspen isolate on sugar maple, each isolate became established and caused decay within vigorously growing trees of aspen,

beech, yellow birch, ironwood, and sugar maple growing under forest conditions (Tables 1 and 2). *Poria obliqua* from yellow birch became established in aspen, beech, birch, and maple. *Polyporus glomeratus* isolated from beech caused decay in beech and birch, the only two species of trees in which it was introduced.

Decay

The amount of decay resulting from inoculation with a single isolate differed greatly between trees of different species (Table 2). The amount of decay that developed within trees of the same species also varied with the different isolates.

These variations in decay between the individual trees over the 10-year

TABLE 2.—*The hosts from which the heartrot fungi were isolated and the vertical extent of heartrot developed in the inoculated trees after 10 years*

Isolate and source	Amount of vertical heartrot in				
	Aspen	Ironwood	Maple	Beech	Birch
	Ft.	*Ft.*	*Ft.*	*Ft.*	*Ft.*
Fomes igniarius					
Aspen	7.09	5.33	0.00[b]	1.91	11.00
Ironwood	3.83	8.06	13.00	... [c]	15.25
Elm	23.00	1.33	18.00	2.50	1.08
Beech	3.00[a]	11.33	6.50	1.41	10.50
Poria obliqua					
Birch	25.00	0.00[b]	0.25	0.66	23.66
Polyporus glomeratus					
Beech	.....	.....	.....	3.50[d]	14.00[d]

[a] Extent of decay at the end of 5 years when tree was accidentally destroyed during cutting operation.

[b] Inoculation failed to take.

[c] Although decay had developed, an extensive bark canker had also formed permitting other decay fungi to enter, making it impossible with certainty to trace decay by *Fomes igniarius*.

[d] Amount of decay in 9 years.

period are to be expected. Individual trees within a species are known to vary measurably in durability (7).

Aspen and yellow birch developed rather extensive decay from both *Fomes igniarius* and *Poria obliqua*. In aspen the decay caused by *P. obliqua* was typical of the decay associated with that fungus in yellow birch.

Fomes igniarius averaged less decay in beech than in any of the other host species (Table 2), although it has been stated that beech in the Adirondacks is severely affected by this fungus (1). It is possible for a fungus to become established readily on exposed dead wood of wounds and still develop slowly in the heartwood of live trees. Much of the infected beech in the Adirondacks is mature or overmature and it is possible for *F. igniarius* to have been present for many years in beech trees.

The failure of the *Fomes igniarius* from aspen to cause decay in the single maple tree does not mean that it cannot become established in sugar

maple. The fungus was dead in the inoculation chamber when the tree was cut but there was no indication why it had died. The surprising fact is that *F. igniarius* became established so readily in the various live hosts following artificial methods of inoculating them.

Sporophore Production

Two fruit bodies of *Fomes igniarius* were produced on the inoculated hosts.

On ironwood inoculated with *Fomes igniarius* from beech, a fertile sporophore became visible in June, 1944, 6 years after the inoculum was planted in the host (Fig. 1, A). The sporophore continued to develop until the tree was cut in 1948. In appearance it resembled the normal sporophore of *F. igniarius,* and the decay associated with it was typical of that produced by this fungus in other hardwoods (Fig. 1, B).

A sporophore began to form by July, 1948, on aspen inoculated with an isolate from aspen (Fig. 1, D). Basidiospores were present when the tree was cut the following October.

DISCUSSION

The fact that *Fomes igniarius* taken from trees of several species of northern hardwoods was established by artificial means in other hardwood species and caused decay, suggests that under natural conditions this exchange of hosts may occur. If this is true, infected stands of aspen in close proximity to other valuable hardwood species may serve as an important source of inoculum and consequent decay. This is especially important because *F. igniarius* may produce fertile sporophores on aspen when the trees are relatively young. During cutting operations in hardwood stands, the removal of all trees infected with *F. igniarius* is a good safety measure.

Poria obliqua is relatively common on yellow birch in the Adirondacks. In this study it caused very limited decay in beech and sugar maple, but very extensive decay in aspen and yellow birch within 10 years. The author is not aware that this fungus has been reported on aspen following natural inoculation. Perhaps the species of hardwoods that are subject to decay by this fungus in America are more extensive than present records indicate.

Polyporus glomeratus isolated from a sterile conk on beech caused decay in beech and birch. Somewhat less than 4 years after the beech tree with a sterile conk was cut and left on the forest floor, fertile sporophores of *P. glomeratus* were produced in great abundance. *Poria obliqua* on birch also produces fertile sporophores several years after an infected tree dies (3). Establishment of sterile conk fungi such as *P. glomeratus* and *P. obliqua* by means of basidiospores is dependent upon the production of fertile sporophores. Therefore during cutting operations it is good practice to remove birch and beech trees bearing sterile conks, and dispose of the infected logs in such a way that the fungi cannot produce the fertile sporo-

phores on the dead wood. This should result in reducing heartrot caused by these fungi.

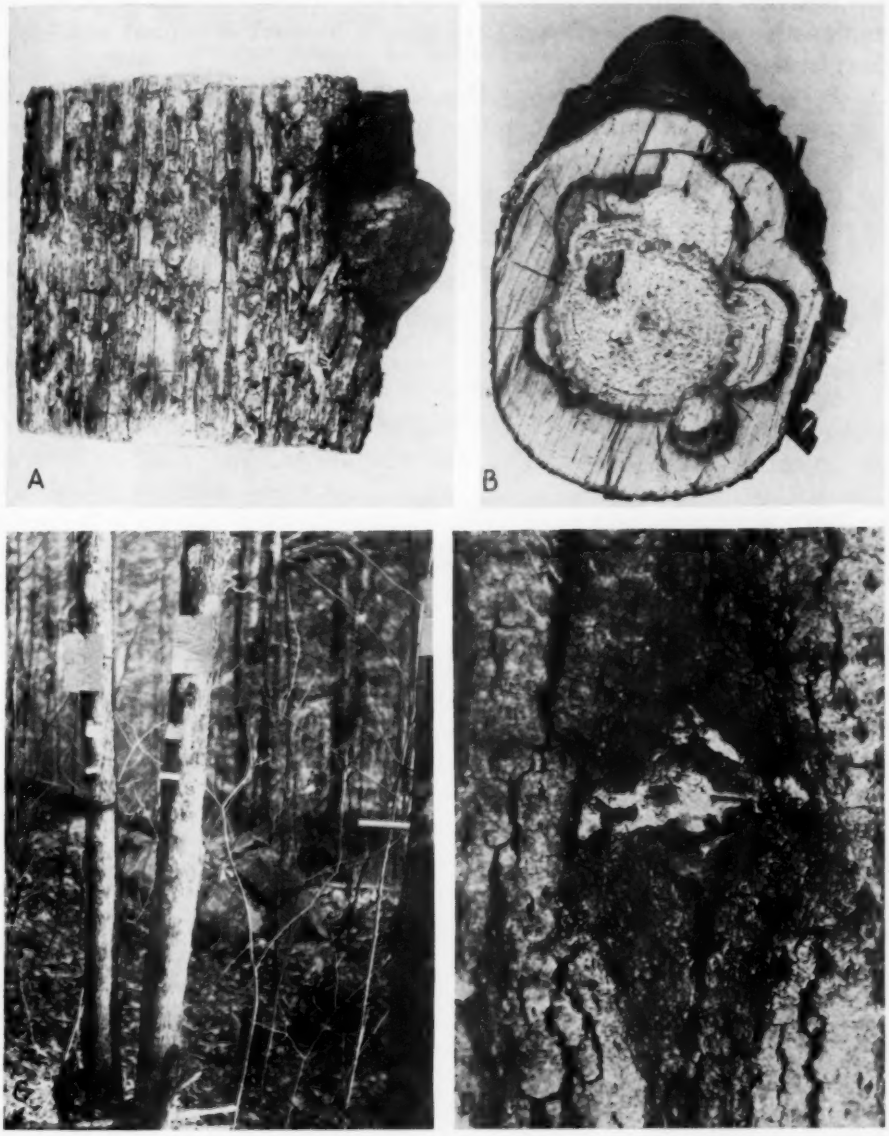

FIG. 1. A. Sporophore on ironwood produced by *Fomes igniarius* isolate taken from beech. B. Cross section of ironwood just above the sporophore showing typical white heartrot. C. Ironwood and sugar maple after inoculation with *F. igniarius* isolates. D. Young sporophore on aspen produced by *F. igniarius* isolate from aspen. (Photographs by J. L. Lowe.)

SUMMARY

Fomes igniarius was isolated from trembling aspen, beech, yellow birch, elm, and ironwood growing in the Pack Demonstration Forest at Warrens-

burg, New York. Forest trees of aspen, beech, yellow birch, ironwood, and sugar maple were inoculated with each of the isolates. Regardless of the host-source of the inoculum, the isolates became established in all of the represented species except one. Appreciable heartrot developed in all the host trees.

Poria obliqua isolated from yellow birch became established and caused extensive decay in aspen and yellow birch, but relatively little decay in beech and sugar maple.

Polyporus glomeratus was isolated from a sterile conk on beech and inoculated into healthy beech and yellow birch. Within both hosts it caused decay.

Sporophores of two isolates of *Fomes igniarius* were produced on two trees: on ironwood in 6 years by a beech tree isolate; on aspen in 10 years by an aspen isolate.

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PIT CANKER OF ELM[1]

NESTOR E. CAROSELLI AND C. M. TUCKER[2]

(Accepted for publication March 3, 1949)

INTRODUCTION

The pit canker disease of elm has been known to arborists for over forty years but its cause has heretofore been undetermined. This abnormal pitted condition of elms has been associated with insects by Stone (4) in whose publication there is a photograph of a pitted trunk. He reported that pitting was often noted on trees growing "under uncongenial conditions."

Other authors have also found that in the majority of cases trees having the disease were growing under poor conditions. Felt and Rankin (2) referred to this disease of undetermined origin as pit canker and reported it to be frequently found affecting elms in New England. A similar canker of elm was described by Fox (3) who contended that the injury occurred as a result of horses chewing the bark of trees. Cankers have been noted occurring on old trees which had rings to which horses were tied. However, cankers on these old trees were observed also at heights of 15 to 20 feet, well out of reach of these animals. This disease is often referred to by arborists as deep pitted canker, deep canker, elm trunk canker (1), and ring canker.

DISTRIBUTION AND ECONOMIC IMPORTANCE

The American elm (*Ulmus americana* L.) is the most susceptible species but the disease has also been found on slippery elm (*U. fulva* Michx.). Samples sent to the Bartlett Tree Research Laboratories for diagnosis indicate that the disease is present in Massachusetts, Connecticut, New York, and Pennsylvania. Dodge (1) reported the disease on trees growing in Montreal, Canada, and in the Middle Atlantic States.

Although the disease has no sudden and drastic effects on the tree, its presence often results in decline. Numerous cankers on the trunk mar the beauty of the tree (Fig. 1, A.). Severely infected trees become unsightly and their removal is necessary. As a rule, the disease is present in a few trees in scattered localities; in certain areas, however, over 60 per cent of the elms are infected. The prevalence of the disease is illustrated by the report of Dodge (1) that within ten years 10 per cent of the elms in a small Massachusetts town became infected.

SYMPTOMATOLOGY

External symptoms of pit canker are the formation of concentrically marked cankers on the trunk and sometimes on the scaffold branches (Fig.

[1] Contribution No. 738 from the Rhode Island Agricultural Experiment Station and the Bartlett Tree Research Laboratories.

[2] Respectively, Associate Pathologist, Bartlett Tree Research Laboratories, in cooperation with Rhode Island Agricultural Experiment Station; and Pathologist, Missouri Agricultural Experiment Station.

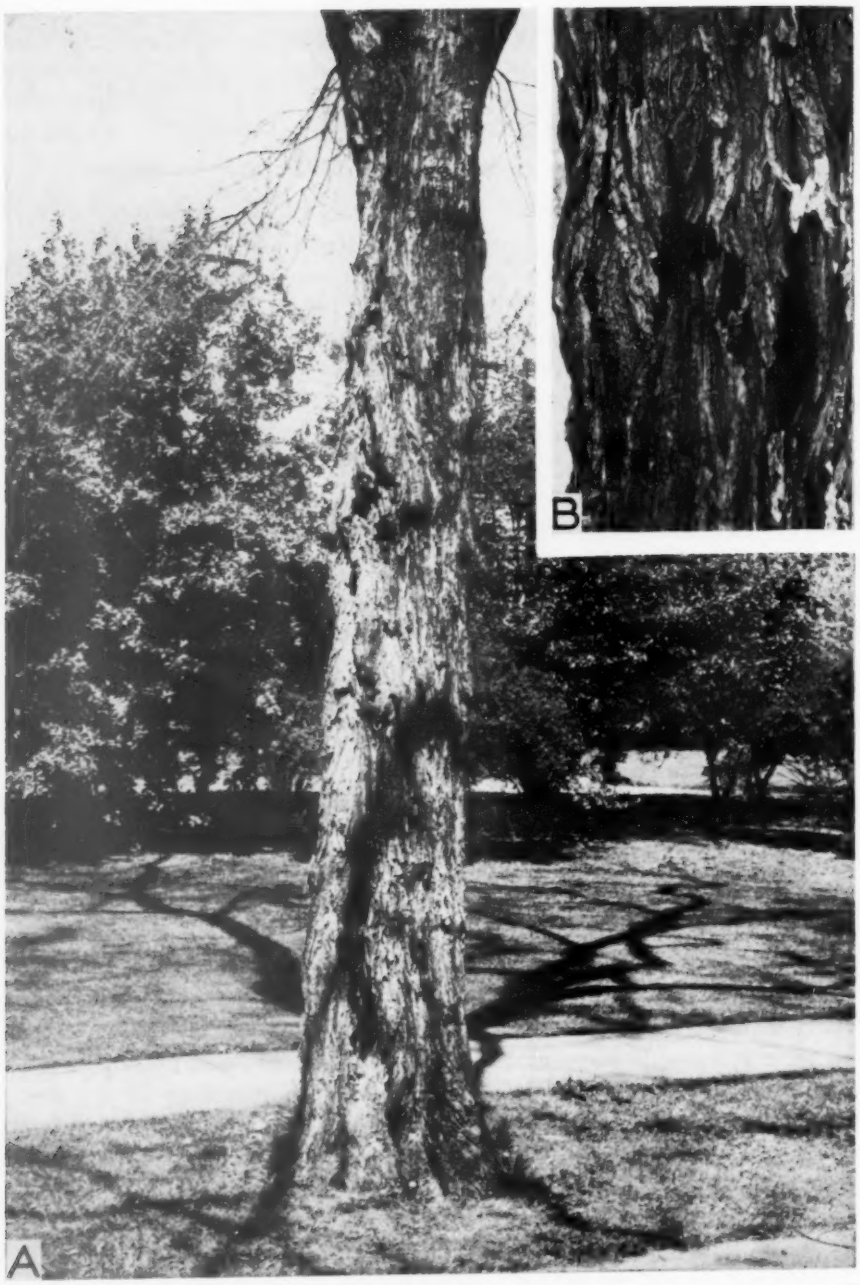

FIG. 1. A. Elm tree showing irregular trunk typical of pit canker disease. B. Close-up of trunk with characteristic pit cankers.

2, B.). The characteristic zoning in the cankers probably represents the yearly progress of the causal organism. After injury is caused by the invading fungus the host develops callus tissue which may partially cover

the injured area. This newly formed tissue is subsequently invaded by the fungus and the concentric ring formation of the cankered area results.

FIG. 2. A. Pit canker development 18 months after inoculation with *Phytophthora inflata* n. sp. Bark has been removed to show the concentric rings formed. B. Old canker showing concentric markings.

Cankers are often masked by the thick bark of the trunk (Fig. 1, B). Bleeding, in the form of a red-brown exudate, often occurs from small fis-

sures near the margin of the cankers. This bleeding is easily distinguished from slime flux in elms which is usually light brown or dark gray. In severely infected trees the trunks become rough and irregular as a result of depressions formed by the cankers and sloughing of the bark near the affected areas. A marked decline of infected trees is indicated by sparse yellow and pale green foliage, reduced terminal twig growth, and, in certain instances, dieback of twigs and small branches.

Internal symptoms are characteristic red-brown discolored tissues in the phloem, the cambium, and the outer area of the xylem. On the trunk where a number of cankers are localized, adjacent cankers are frequently connected by strands of discolored, infected tissue. Where cankers are widely separated there does not appear to be any connection between them.

THE PATHOGEN

Investigations on the disease were initiated in the spring of 1946 when tissues collected from declining trees growing in various sections of Connecticut and Massachusetts yielded a *Phytophthora*. Twenty-five street trees in Greenwich, Connecticut,[3] each with typical cankers, were used as sources of material at intervals through the spring and summer.

On May 5, 1946, samples taken from the 25 diseased trees were cultured on apple-potato-dextrose agar. The organisms that developed from the transplanted tissues were examined periodically. After 14 days only two samples yielded *Phytophthora*. Samples were collected again on June 4, 1946, from those trees which were negative for *Phytophthora,* and four more trees were found infected. On June 22, 1947, three additional cases were found; on September 10, 1947, four cases proved positive and on October 11, 1947, two more trees yielded the pathogen. Finally the *Phytophthora* was isolated from 15 of the 25 cankered trees.

Difficulty in isolating the fungus on agar media resulted from the presence of contaminants such as *Alternaria* sp., *Monilinia* sp., *Rhizopus* sp., and *Fusarium* sp. These fungi grew rapidly and often masked the presence of the slow-growing *Phytophthora*. Isolation of *Phytophthora* was most successful when the inoculum was plated on autoclaved applesauce. The samples to be cultured were dipped in alcohol and quickly flamed. Excessive heat was avoided by using rapid recurrent flamings of short duration. After flaming, the samples were placed on autoclaved applesauce in Petri dishes. When a fungus growth was noted during daily examinations with a dissecting microscope, mycelium was transferred to apple-potato-dextrose agar in another Petri dish. The transferred organism was also observed closely and as soon as a coenocytic mycelial strand was observed, it was immediately transferred to potato-dextrose agar. On apple-potato-dextrose agar the fungus develops a compact brown circular pad of mycelium adhering closely to the substrate. This characteristic has aided in iso-

[3] Thanks are expressed to Mr. Joseph Dietrich, Tree Warden of Greenwich, Connecticut, for his cooperation in certain phases of this work.

lation, particularly when various contaminants overran the surface of the medium.

Isolates obtained from trees growing in Massachusetts, Connecticut, and New York proved indistinguishable in morphologic and cultural characters. The fungus differs in important respects from previously described species of the genus and is here designated a new species.

Phytophthora inflata sp. nov.[4]

Hyphae continuous when young, becoming septate with age, usually smooth. Sporangia rare on agar media but developing abundantly on washed mycelial mats transferred from 7-day pea broth cultures to sterile distilled water and incubated 7 days; sporangia borne terminally on sporangiophores not differentiated from vegetative hyphae, limoniform to elongate, papilla broad, rounded and nearly filled by contents of sporangium; hyaline dehiscence plug lenticular; sporangia variable in shape and size, 20–67 × 15–32 μ, averaging about 38 × 23 μ. Zoospores fully differentiated within the sporangium, biflagellate, reniform in motile stage, spheroidal in quiescent stage, 10–12 μ in diameter. Chlamydospores not observed, but thin-walled, hyaline intercalary hyphal swellings occasionally develop. Oogonia, abundant in oatmeal agar cultures, thin-walled, hyaline, spheroidal, stalk narrow, not infundibuliform, 30–42.7 μ, averaging 34 μ. Oospores single, spheroidal, smooth, nearly filling oogonium, hyaline to straw color, wall fairly thick (3–4 μ), contents densely granular, usually with a single large reserve globule and frequently 1 to 4 smaller globules, 26–39.3 μ, averaging 31.3 μ. Antheridia paragynous, point of contact with oogonium usually near oogonial stalk, inflated, usually variously contorted, often twining or twisted about oogonial stalk, often irregularly lobed or branched, arising from a separate hypha or, occasionally, from oogonial hypha, more numerous in culture than oogonia but seldom more than one in contact with an oogonium, up to 50 μ long and 15 μ in diameter.

Habitat—living cortical tissues of *Ulmus americana* L. and *U. fulva* Michx., northeastern United States of America.

Type cultures have been deposited in the American Type Culture Collection and at the Centraalbureau voor Schimmelcultures.

Hyphis primo continnis, maturitate septatis, vulgo levibus.

Sporangiis in agare cultis raris, sed copiosis in mattis mycelialibus lavatis e solutione nutritia septem dierum ad aquam distillatum et sterilem translatis et septum dies incubatis; nascentibus in sporangiophoris non a mycelio distinctis; vulgo limoniformibus, elongatis, papillatis, 20–67 × 15–32 micra, fere circa 38–23 micra.

Zoosporis in sporangia formatis, biflagellatis, motis concavo-convexis, quietis globosis, in diametrum 10–12 micra.

Chlamydosporis ignotis.

Oogoniis spheroidalibus, hyalinis, levibus, tenuimembraneis, cauli angusto, 30–42.7 micra, fere 34 micra. Oosporibus singulis, levibus spheroidalibus hyalinis aut subfuscis, 26–39.3 micra, fere 31.3 micra. Membrana crassa (3–4 micra). Antheridiis paragynosis, inflatis, vulgo multis modis contortis, non constanter lobatis aut ramosis.

[4] Acknowledgment is made to Prof. W. E. Gwatkin, Professor of Classical Languages and Archaeology, University of Missouri, for assistance in preparation of the Latin description.

Habitat in cortice vivo *Ulmi americanae* et *U. fulvae* in regione U. S. A. vergente in septentriones et orientem solem.

Phytophthora inflata grows well on the usual agar media. On Difco corn-meal agar (pH 6.0) the temperature-growth relations of the species indicate an optimum temperature between 25° and 30° C. Growth of Petri-plate cultures after 96 hr. was as follows: at 35° C.—no growth, inoculum killed in 96 hr.; at 30° C.—33 mm.; at 25° C.—34 mm.; at 20° C.—29 mm. Oogonia and antheridia develop abundantly on cornmeal agar but are somewhat smaller than those developing on oatmeal agar. The species resembles *P. megasperma* Drech. and *P. erythroseptica* Pethyb. to a degree in the large size of oogonia and oospores. It may be distinguished from *P. erythroseptica* by its paragynous antheridia, and from *P. megasperma*, as from all other species, by the large, inflated, contorted, variously lobed or branched antheridia which are considered the distinguishing character of the species (Fig. 3, 9–17). The antheridia may be observed most favorably in young cultures prior to, or soon after, fertilization of the oogonia. In older cultures they are often tightly twisted around the oogonial stalk and difficult to see.

The character of the sporangia developed in distilled water is not well marked. The broadly rounded papilla with its rather thin or lenticular hyaline plug suggests the non-papillate sporangia characteristic of *Phytophthora cryptogea* Pethyb. & Laff., *P. cinnamomi* Rands, and closely related species. Sporangia developed in oatmeal agar cultures, though few in number, have the well-defined hyaline plug similar to those formed by *P. parasitica* Dast., *P. palmivora* Butl., and *P. capsici* Leon.

In apple fruits inoculated by incision, *Phytophthora inflata* caused a firm, rapidly spreading brown rot. Approximately half of a potato tuber was invaded by the fungus within one week. When the invaded potato tissue was exposed to the air a bright pink color developed within a few minutes. The brown rot of apple fruits and the pink rot of potato tubers were similar to rots caused by numerous species of the genus (5).

PATHOGENICITY TESTS

The pathogenicity of the *Phytophthora* was tested on 27 elms ranging from 3 to 17 in. in diameter and growing on low wet and on high dry sites. Inoculations were made by inserting mycelium from active cultures into vertical wounds made in the trunks with a flamed scalpel. Sterile media and the fungi mentioned previously as contaminants were placed in wounds on trees as a check on symptom development. In 1946, five trees were inoculated on August 5; five on August 20; four on September 15; nine on October 15; and four on October 22. About five weeks after the first three groups were inoculated, symptoms began to appear in 81 per cent of the trees, while the checks remained apparently healthy. The first noticeable symptom was the presence of a soft, dark brown exudate near the point of inoculation. As the season progressed the underlying tissue became ne-

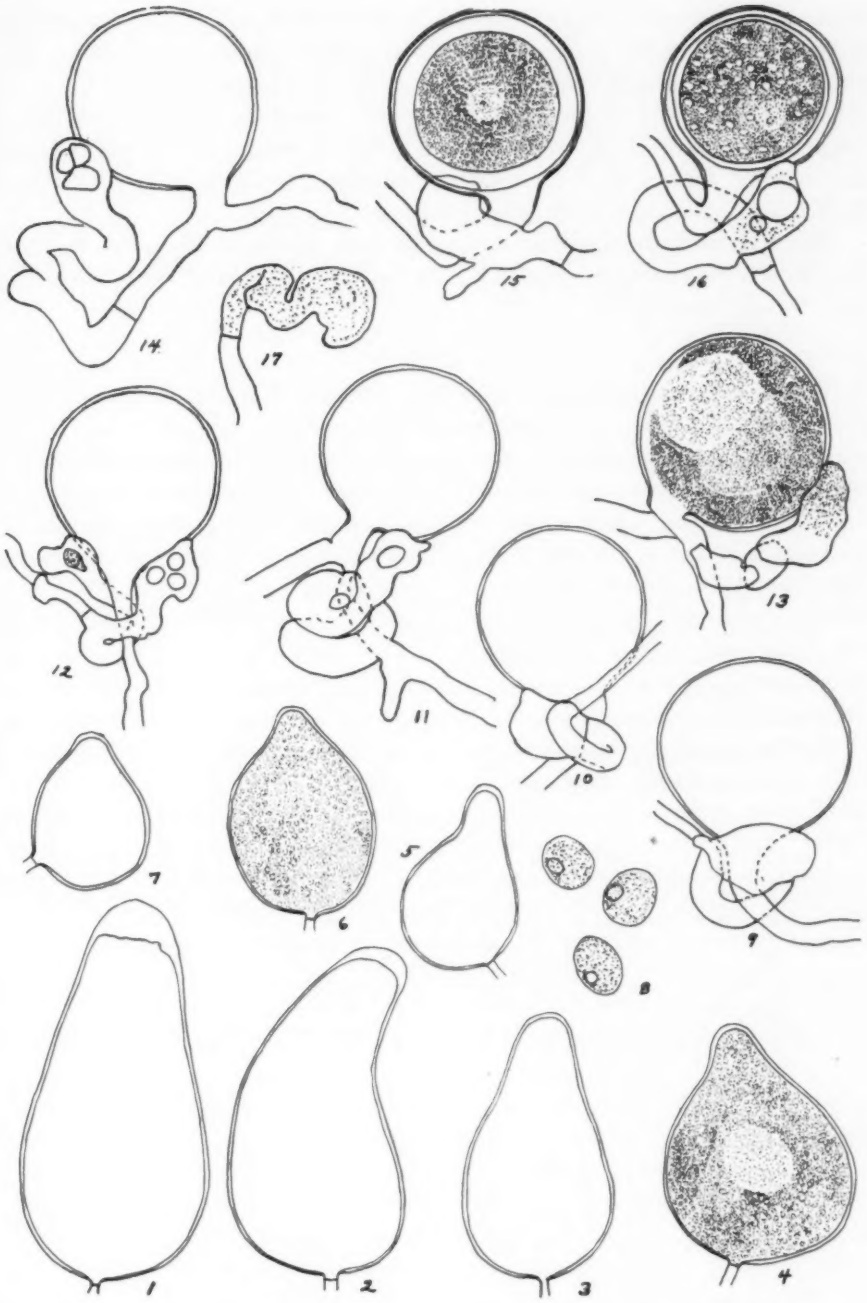

FIG. 3. Phytophthora inflata n. sp. 1–7, Sporangia developed on washed mycelium transferred from pea broth to distilled water; 8, zoospores; 9–14, oogonia and antheridia; 15, 16, oogonia, oospores, and antheridia; 17, antheridium. (×1000)

crotic and cankers developed similar to those from which the fungus was isolated. As new tissue developed, it was killed back by the fungus. This produced the concentric zoning characteristic of the cankers (Fig. 2, A). In the spring of 1947, 83 per cent of the inoculated trees had symptoms of canker. Removal of the bark of the newly formed cankers showed that the infected tissues were immediately adjacent to the cambium and were dark brown as in naturally infected trees.

Reisolation from diseased tissues at some distance from the point of inoculation consistently yielded a *Phytophthora* identical with the isolate used for inoculation.

SUMMARY

Pit canker of the American elm occurs widely distributed in New York, Pennsylvania, Connecticut, and Massachusetts.

Phytophthora inflata n. sp. was associated with the cankers. The causal relationship of the fungus was established according to Koch's postulates.

Phytophthora inflata n. sp. is readily identified in culture by the development of large, inflated antheridia, which are usually variously contorted, lobed, or branched.

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FORMATION OF GALLS IN STEMS AND LEAVES OF SUGAR CANE IN RESPONSE TO INJECTIONS OF GROWTH-REGULATING SUBSTANCES[1]

J. GUISCAFRÉ-ARRILLAGA

(Accepted for publication March 8, 1949)

During the late summer and fall of 1947 and 1948, experiments were undertaken at Baton Rouge, Louisiana, to test the effect of various growth-regulating substances upon the inducement of floral organs in sugar cane. Sugar cane seldom blooms in this area, and when it has bloomed, only a few tassels have formed in a whole field, thus indicating that conditions in this region are not quite favorable for flowering.

Naphthalene-acetic acid, indole-acetic acid, and 2,4-dichlorophenoxyacetic acid were injected approximately ½ in. above and ½ in. below the terminal bud in amounts of 0.5, 1.0, 1.5, 3.0, and 4.0 mg. Solutions of these chemicals were prepared so as to contain 1 mg. per cc. Concentrations used were based on those found by Van Overbeek (8) to induce flower formation in the pineapple plant. Ten stalks of C.P. 29/116 were injected with different amounts of each of the three chemicals. Stalks of this variety were also injected with 0.5 cc. of a solution containing 0.5 mg. of naphthalene acetic acid and 0.5 mg. of colchicine per cc. Similar tests were conducted in the same field at a distance of 200 yd. using the variety C.P. 29/320. In both cases, plants were treated also with 0.5, 1, and 1.5 cc. of sterilized distilled water. To serve as controls, a number were punctured with the needle and air was blown into the tissues. Tests were started on August 15. Different lots of stalks were injected with varying dosages of each chemical. The concentrations of 1.0 and 1.5 mg. were divided for some lots into two or three portions of 0.5 mg. each and injected at 2-wk. intervals. For amounts of solution such as 0.5 and 1.0 cc., the short type 1-cc. Yale Insulin Hypodermic Syringe No. 1Y1–80 with needle size Bd 27 was used; for larger quantities, the 5 cc. B-D Yale Hypodermic Syringe No. 5Y.

The needle was thrust at an angle of 30–40° until it had penetrated through half of the diameter of the stalk at the region where the terminal bud was located. The liquid could not be forced into the tissue unless the needle was drawn out about 2 mm. It often was necessary to free the needle of plugging material after each injection. Both needle and syringe were thoroughly cleaned several times by flushing with 95 per cent ethyl alcohol and sterilized distilled water, when changing to another chemical.

With the exception of observations on the technique of treatment, no

[1] The author wishes to acknowledge the assistance given by Dr. C. W. Edgerton, Head of the Department of Botany, Bacteriology, and Plant Pathology, and by Drs. Lewis H. Flint, Charles F. Moreland, and M. T. Cook of the staff of the same department, in conducting and presenting the experiments herein reported.

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FIG. 1. Single stalk on left: sprouting of lateral buds induced by injecting 3–5 mg. of indole-acetic acid. Three stalks on right: galls induced by injecting stalks with 0.5 mg. of indole-acetic acid three times at intervals of 2 weeks.

results were obtained from the 1947 experiments, yet it proved feasible to introduce as much as 5 cc. of liquid near the terminal bud without apparent injury. Further observations were not possible because the lot was har-

vested before any reasonable length of time had elapsed after injection of the chemicals. Observations made between November 1 and December 31, 1948, showed no initiation of floral organs in any of the stalks treated.

However, some very interesting responses to the injection of various amounts of the growth-regulating substances at the terminal bud were observed. With larger amounts of indole-acetic acid such as 3 and 4 mg., the newly formed internodes grew at an angle in a zigzag fashion and the buds sprouted into shoots, the terminal bud remaining unaffected.

FIG. 2. Two stalks on left: gall formation induced by injection of 0.5 mg. of 2,4-D. Two stalks on right: gall formation induced by injection of a mixture of 0.5 mg. of naphthalene acetic acid and 0.5 mg. of colchicine.

Amounts of indole-acetic acid corresponding to 1.5 mg. divided into 3 portions of 0.5 mg. each and injected at 2-wk. intervals caused the formation of galls in the more mature internodes close to the top of the stalks. These galls were formed both at the nodes and on the internodes and gave origin to buds growing in all directions, both at the internodal and nodal regions (Fig. 1). These galls were not of a folioid nature but rather firm, pinkish, and bud-like. Out of 10 stalks thus treated, 5 showed these galls profusely, while other stalks in the vicinity, and some originating from the

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same clump but treated differently or receiving no treatment at all, had no gall formation. The galls resembled those on sugar cane as reported by Lyon (3), Martin (4, 5), and Tims (7).

When applied in amounts of 0.5 mg., 2,4-dichlorophenoxyacetic acid induced gall formation at the base of the youngest leaves visible in the spindle and in the covered immature internodes. The galls in the leaves were small and scabby, while those in the covered immature internodes were large, tender, and distinctly folioid (Fig. 2). When mixed in the same solution, 0.5 mg. each of naphthalene acetic acid and colchicine induced the same type of folioid galls in covered immature internodes in 4 of the 10 stalks treated (Fig. 2).

None of the responses described was observed in any of the other treatments nor in stalks injected with water, or simply punctured or not treated at all. Untreated stalks of other varieties in the vicinity of the treated plots showed no gall formations. The infestation of the borer was great in all the plots and to the same extent in treated and nontreated stalks.

Galls have been observed in sugar cane by several investigators including Kamerling (2), Barnum (1), Lyon (3), Martin (4, 5), and Tims (7). Tims (7) obtained galls in sugar cane by planting cuttings from stalks of P.O.J. 234 previously showing gall formation or showing symptoms of the dwarf or multiple bud disease. Martin (5, 6) reported the inducement of gall formation in sugar cane by injections of extracts obtained from crushed green leafhopper, *Draeculacephala mollipes*, corn leafhopper, *Peregrinus maidis*, and pink sugar cane mealy bug, *Tryonymus sacchari*. He suggested that the green leafhopper as well as the other insects might carry certain auxins or growth-promoting substances which induced gall formation.

The findings hereby reported support Martin's (5, 6) observations and theory as to the etiology of galls in sugar cane as well as that of Went and Thimann (9) on the cause of pathological outgrowths of plants as being traceable to auxin effects.

This seems to be the first instance in which galls have been induced in sugar cane by the artificial injection of growth-regulating substances, and offers a new and practical method of stimulating the formation of excessive proliferated tissues which may give origin to polyploid plants.

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REPORT OF THE 1949 ANNUAL MEETING OF THE SOUTHERN DIVISION, THE AMERICAN PHYTOPATHOLOGICAL SOCIETY

The 1949 annual meeting of the Southern Division of The American Phytopathological Society coincided with that of the Association of Southern Agricultural Workers, January 31 through February 2, 1949, at Baton Rouge, Louisiana. More than 90 plant pathologists were in attendance. In addition to the 34 papers presented, a round-table discussion on the plant nematode problems of the South was held, led by Dr. G. Steiner of Beltsville, Maryland.

Officers elected for 1949–50 are: *President*, L. M. BLANK; *Vice-President*, W. A. JENKINS; *Secretary-Treasurer*, J. A. LYLE; *Councilor*, A. L. SMITH.

Titles and abstracts of papers presented follow.

J. A. LYLE, *Secretary-Treasurer*

Fungicides and Fall Cucumbers in Louisiana. ATKINS, J. G., JR. In Louisiana the basis for evaluation in the testing of fungicides for fall cucumbers has shifted in recent years from one based upon the control of downy mildew to one based upon the control of two foliage diseases, downy mildew and anthracnose. Although downy mildew still ranks as the principal foliage disease, the frequent severity of anthracnose necessitates a fungicide effective against both diseases. The testing of organic and fixed copper fungicides has been complicated by the varying severity of anthracnose from year to year. In 1947, a year of heavy anthracnose infection, Tri-Basic Copper Sulphate gave poor performance as a cucumber fungicide in comparison with the organic fungicides. However, the same fungicide gave good results in 1948 when anthracnose was not severe. In both the 1947 and 1948 tests Dithane Z-78 (zinc ethylene bisdithiocarbamate), Fermate (ferric dimethyl dithiocarbamate), Zerlate (zinc dimethyl dithiocarbamate), and Parzate (zinc ethylene bisdithiocarbamate) gave good control of both downy mildew and anthracnose. Dithane Z-78 was not phytotoxic on cucumbers but Parzate caused considerable injury.

Varietal Response of Snap Beans to Seed Treatment. BAIN, DOUGLAS C. Studies of seed treatments for control of seed-rot and damping-off of snap beans were made in the field at Crystal Springs, Mississippi. The materials used were Spergon (98 per cent tetrachloro-para-benzoquinone), Arasan (50 per cent tetramethyl thiuramdisulfide), Arasan SF (75 per cent tetramethyl thiuramdisulfide), Ceresan M (ethyl mercury p-toluene sulfonanilide), Semesan (30 per cent hydroxymercurichlorophenol), Puratized 177 (phenyl mercury triethanolammonium lactate), and 2 per cent Ceresan (2 per cent ethyl mercury chloride). The materials were applied as dust or slurry or both. Plantings were made in both spring and late summer. Results indicated that in spring planting the variety Stringless Black Valentine responded significantly to some treatments in increase of emergence, stand, and yield; however, under like conditions Fulgreen 1 responded only slightly. In late summer plantings of Stringless Black Valentine there was a significant increase in emergence and stand but not in yield; the response of the U. S. Dept. Agr. line B1625-17, however, was negative; *i.e.*, emergence, stand, and yield were reduced in most cases, while plants from untreated seed had the highest yield. These differences in response did not appear to be due to resistance to seed-rotting and damping-off organisms, if the percentage of loss due to these causes is used as the criterion. Certain treatments among these varieties appeared to increase emergence and stand but decreased yield as compared with untreated seed.

Breeding for Resistance to Bacterial Blight of Cotton. BLANK, LESTER M. Stoneville 20 strain of cotton has been used as the blight resistant parent in a number of crosses with susceptible varieties and breeding strains of cotton. The resulting progenies were carried through the F_3 generation or have been backcrossed twice to the recurrent susceptible variety or strain, selfed, and tested for disease reaction. Inoculation of the breeding material in field plantings was accomplished by spraying the underside of leaves of 5- to 8-week-old plants with a suspension of *Xanthomonas malvacearum.*

Grading of plants as to disease reaction was made 15 to 20 days later. Resistant phenotypes have fewer and smaller lesions than do susceptible phenotypes. F_1 progenies of the original cross were susceptible while F_2 progenies segregated at a ratio approximating 3:1 for susceptibility and resistance. This ratio was realized most closely in crosses involving highly susceptible varieties such as Acala, and less closely in crosses involving varieties having considerable natural tolerance. Progenies derived from selfing after one and two backcrosses gave populations which were pure for susceptibility or were segregating, the segregating populations having susceptible and resistant plants in a ratio of 3:1. These results suggest that the Stoneville 20 type of resistance to bacterial blight is inherited as a single factor, with resistance recessive to susceptibility.

Hypodermic Injection as a Method of Inoculating Cotton Plants with Verticillium albo-atrum. BRINKERHOFF, L. A. In breeding for resistance to Verticillium wilt of cotton much time is usually spent selfing plants that prove to be susceptible later in the season. In California and Arizona rarely more than 15 to 30 per cent of a susceptible population will show symptoms at the start of the selfing season. This is true for both naturally infested or artificially inoculated soil. Hypodermic injection and wound inoculation both produced high percentages of infection in young field-grown cotton plants in 1947 and 1948. The injection method proved to be about as rapid as soil inoculation and much faster than wound inoculation. Seedlings with two to four true leaves were much easier to inject than older plants. A veterinary syringe with a No. 24 needle was used to inject approximately ¼ ml. of inoculum into the hypocotyl of young seedlings just below the soil level. Ninety-nine and 98 per cent respectively of a susceptible variety, Acala 1–23–11, and a tolerant variety, Missdel × Acala 10–13, developed symptoms within 3 weeks after injection on May 17, at Shafter, California. The tolerant variety was much less severely infected, and a much higher percentage of the plants recovered from the disease.

Induced Variability in Phoma lingam. CALVERT, O. H., GLENN S. POUND, J. C. WALKER, MARK A. STAHMANN, and J. F. STAUFFER, JR. Induced variability of the Puget Sound strain of *Phoma lingam* recently described as occurring on cabbage seed plants has been studied in relation to a sudden development of seed transmission on Puget Sound seed. In determining whether this blackleg epidemic resulted from increased activity of the Puget Sound strain, nitrogen mustard (methyl-bis (B-chloroethyl) amine) was used to induce cultural and pathogenic variability. It appeared that the rate of mutation could be markedly increased by appropriate treatment and the nitrogen mustard appeared to be the most effective means used by which mutations could be induced. In one experiment over 60 per cent of mutations occurred among surviving spores. There was apparently no correlation between the type of mutant produced and the method of treatment used. Greenhouse inoculations were made to determine the extent of induced pathogenic variability by nitrogen mustard. No isolate produced lesions characteristic of those of the eastern type strains, but evidence was presented to show that through increased virulence some of the isolates were able to produce a disease as severe as that caused by the eastern strains. Complete evidence was not found, however, that would explain the sudden development of blackleg transmission on western-grown seed.

The Nature of Resistance in Plants to Root-Knot. CHRISTIE, J. R. The root-knot nematode, *Heterodera marioni*, is a sedentary parasite. It remains in the same location throughout the parasitic phase of its life and feeds by puncturing cells within reach of its head and sucking out their content. In a suitable host, pronounced morphological and physiological changes in the surrounding plant tissues, including the formation of so-called "giant cells," provide the parasite with a continuing source of accessible food. It is suggested that, in the case of resistant plants whose roots are invaded but in which larvae fail to develop, changes in the surrounding plant tissues, necessary for the development of the parasite, fail to take place and the nematode dies of starvation.

The So-Called Live-Oak Disease in Texas. DUNLAP, A. A., and A. L. HARRISON. The dying of live oaks in certain areas of Texas has become a serious problem for home owners and ranchmen. Both large and small trees die at different seasons and under a wide variety of environmental conditions. As yet, no causal agent has been identified in connection with this trouble, which is presumed to be some sort of disease. Timely, severe pruning of all large branches at the first appearance of the trouble has resulted in apparent recovery and renewed growth from the main trunk of the tree.

Fungicides for the Control of Raspberry Diseases. EPPS, JAMES M., and C. D. SHERBAKOFF. Of several fungicides tested for the control of raspberry diseases, Fer-

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mate (ferric dimethyl dithiocarbamate) proved most effective when used at the rate of 2 lb. in 100 gal. of water. A delayed dormant spray of 1 to 10 lime-sulphur applied at the time the buds are breaking and three applications of the Fermate spray gave a substantial increase in yield over the other treatments. The copper sprays and dusts did not give an increase in yield over the plot that received the delayed dormant spray alone.

Pecan Scab Spray Experiments in South Carolina During 1948. FOSTER, H. H., A. M. MUSSER, R. J. HIGDON, and M. B. HUGHES. Experimental spray plots, using the scab (*Cladosporium effusum*) susceptible Schley variety, were located near Orangeburg and at the Edisto Experiment Station. Bordeaux Mixture (4–1–100) for first cover spray, and (6–2–100) for second, third, and fourth cover sprays, Zerlate and Karbam White (zinc dimethyl dithiocarbamate) (2 lb.–100), were used at the Edisto Station. At the Orangeburg plots Bordeaux Mixture, Copper-A (3lb.–100), Zerlate, Parzate (zinc ethylene bis dithiocarbamate) (2 lb.–100), and a Cu.-Zn. compound, mixed for pecan spraying (5 lb.–100), were used. Scab infection was rated from one (no infection) to five (numerous secondary infections). Unsprayed check trees showed 77 to 99 per cent of nuts examined to be severely scabbed (class 5). At the Edisto Station Bordeaux Mixture gave practical control of scab and increased the size and weight of nuts, while Zerlate and Karbam White failed to give satisfactory scab control. At the Orangeburg plots all sprays except the Cu.-Zn. compound gave practical scab control and increased the size and weight of nuts. Zerlate was superior to other treatments. At the Orangeburg plots, in part because of insufficient rainfall, some nuts from all treatments including the control had split shells. Bordeaux-sprayed nuts had more split shells than did those under other treatments.

Fungicidal Control of Cercospora Blight of Pepper. HARE, W. W., D. C. BAIN, and T. E. ASHLEY. A field of pepper almost completely defoliated by *Cercospora capsici* was found in the canning area near Wiggins, Mississippi, on July 23, 1948. Spray and dust treatments were applied in replicated plots in the field on that date and approximately every 2 weeks afterward until the end of September. One month after final application a disease rating of the plants was made, based on the percentage of defoliation of the second crop of leaves. Analysis of indexes calculated for each treatment plot showed highly significant effects of Bordeaux (4–4–50) and Copper A sprays as compared with the dusts in the prevention of defoliation. Copper A and Tribasic Copper dusts were highly significantly better than the checks. There were no significant differences between the two sprays or the two dusts. The sprays gave satisfactory control of the disease. The control obtained with the dusts was not considered sufficient for practical use.

Some Factors Affecting Viability of Peanut Seed. HIGGINS, B. B. Most of the late-harvested peanuts of the 1947 crop in the southeastern area of the United States were severely weather-damaged; consequently, the viability was low. Stands obtained from early plantings were generally poor. In an attempt to clear up some questions as to the effect of various chemical seed-treatment materials, variation in rate of application, and of weather conditions, tests were set up using farmers' stock Runner and Spanish peanuts which were machine-shelled and treated with Dow 9B (50 per cent zinc trichlorophenate), 2 per cent Ceresan (2 per cent ethyl mercury chloride), Arasan (50 per cent tetramethyl thiuramdisulfide), or Spergon (98 per cent tetrachloro-para-benzoquinone), all at normal, medium-high, and high rates of application and with machine-shelled and hand-shelled checks. Two plantings of the Runner seed were made, one when conditions for germination were nearly ideal and the other just before a series of heavy rains. The Spanish were planted under nearly ideal conditions for germination. Both Runner and Spanish were low in viability and both were severely damaged by machine shelling. There was no noticeable indication of injury from the highest rate of application (6 oz. to 100 lb. of seed) for any of the chemicals. At corresponding rates of application, 2 per cent Ceresan was significantly superior to all other materials, except at 6 oz. to 100 lb. of Spanish seed when the superiority to Arasan and Spergon was not significant. The Runner seed planted just before a series of heavy rains lost more than 32 per cent in viability. Studies and observations for several years indicate that this may be due to loss of a substance from the seed coat, probably the pigment, which appears to have antioxidant and possibly fungistatic properties.

Baldulus Maidis, Leafhopper Vector of Corn Stunt Virus, in Texas. HILDEBRAND, E. M. Adult, light-colored leafhoppers collected near Weslaco, Texas, on May 1, 1948, were placed on small caged field-corn plants for study at College Station. Within 4

days wilt-blight necrotic symptoms developed on the small plants receiving the light-colored leafhoppers, whereas no symptoms developed on plants receiving darker colored leafhoppers. The symptomed plants survived when the leafhoppers were promptly removed. On the 5th day all of the latter (dark-colored) leafhoppers were dead whereas two of the former were alive; these were transferred to healthy caged corn seedlings. On the 6th day some early instar nymphs were found on the seedlings first fed on by the leafhoppers. By the 22nd day the first adults appeared and on the 24th numerous adults were present. Thus the life cycle had been completed within about 3 weeks. Adult specimens of the first brood were identified by Oman as *Baldulus Maidis*. Transfers of leafhoppers were made successively at short intervals to small seedlings for about 3 months. The adult-to-adult leafhopper generation period ranged between 18 and 24 days. Essentially the same generation time obtained for leafhoppers present on diseased plants. The shortest incubation interval between feeding on diseased corn and actual transmission was approximately 24 days.

Fungus Gall on Jasmine. HILDEBRAND, E. M. Gall specimens simulating crown gall on above-ground stems of Winter Jasmine, *Jasmium nudiflorum*, were received from Arlington in October, 1948, and isolations were made. The interiors were sterile whereas the surface tissues yielded a fungus by both tissue-plating and dilution techniques. Abundant black pycnidia, measuring 77 to 98 μ in diameter, covered the gall surface. The radial extension of mature galls ranged between 6 and 12 mm. The fawn-gray cultures produced pycnidia on potato-dextrose agar. Spray inoculation of wounded and unwounded plants of two species of Jasmine and Privet resulted in most abundant infections on wounded plants receiving a post-inoculation high-humidity treatment. Simple wound inoculations were also successful. Gall symptoms were appearing within 2 weeks, with radial extensions of about 1.5 mm. at 1 month and 3 mm. at 2 months. These Jasmine galls were very similar to the so-called Phomopsis galls reported by Brown from Maryland in 1936 and observed by the writer in New York on wild rose in 1937 and on forsythia in 1938. Pycnidiospores are very small, about 3.4×1.5 μ. Based on its small spore size and its lack of *b* spores, this fungus would appear to be a new Phoma species.

Grafting Methods Used in Attempts to Transmit the Littleleaf Disease. JACKSON, L. W. R., and BRATISLAV ZAK. The systemic nature of the littleleaf disease of shortleaf pine (*Pinus echinata*) suggested that it may be caused by a transmissible virus. The transmissibility of littleleaf was tested by grafts made on seedling, sapling, and adult shortleaf pines. The grafting methods are described and illustrated. Stem, branch, and root parts were successfully grafted by the bark patch and the approach graft methods. Much of the difficulty involved in grafting was due to the age of the scion and stock material, because the littleleaf disease does not affect trees under 20 years of age. Positive fusions with bark patch grafts in all except 3 of the special types of grafts ranged from 19 to 100 per cent. Bark patch grafts made with a thin layer of underlying wood or with attached twigs were not successful. Positive fusions with seedling stems grafted on stems of diseased and healthy trees were 78 and 83 per cent, respectively. Positive fusions with seedling roots grafted on roots of diseased and healthy trees were 55 and 36 per cent, respectively. Thus far, the various types of grafts have failed to demonstrate that the littleleaf disease is caused by a virus.

Pathogenicity Studies with Colletotrichum Isolates from Sugar Cane, Sorghum, and Other Crosses. LEBEAU, F. J. Three hundred and seventy-six isolations of *Colletotrichum* from 17 different hosts, the majority coming from sugar cane, sorghum, Johnson grass, Sudan grass, and *Erianthus sp.*, have been tested for pathogenicity towards sugar cane (red rot in variety C. P. 29/116) and sorghum (leaf spot on variety Collier). Of these, 50 cultures were also tested for ability to produce stalk rot in sorghum variety Rex growing in the field. An additional 197 cultures from Johnson grass, sorghum, and sugar cane were tested for their ability to produce stalk rot in sugar cane and sorghum. Using disease ratings from 0 to 4, with 4 indicating greatest severity of disease, the average ratings were 3.58, 1.55, 2.17, 1.46, and 1.78 for sugar cane inoculated with cultures from sugar cane, sorghum, Johnson grass, *Erianthus sp.*, and Sudan grass, respectively. Punctured noninoculated controls yielded an index of 1.11. Sorghum seedlings, inoculated in the leaf whorl with cultures from sugar cane, sorghum, Johnson grass, *Erianthus sp.*, and Sudan grass yielded average ratings of 1.79, 3.44, 2.96, 3.16, and 3.11, respectively. In standing Rex sorghum, isolates from sugar cane, sorghum, and *Erianthus sp.* produced ratings of 1.91, 2.80, and 2.49, respectively. In cut Rex sorghum, cultures from sugar cane, sorghum, and Johnson grass yielded ratings of 1.88, 3.47, and 3.47, respectively. Punctured controls yielded an index of 1.17. Thus the Colletotrichum population on sugar cane is different from that affecting sorghum, Johnson grass, *Erianthus sp.*, and Sudan grass.

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Soil Fumigation for the Control of the Root-Knot Nematode in Peach, Fig, and Grape Plantings. MACHMER, JOHN H. Planting sites at Tifton, Georgia, fumigated in January, 1944, at 6- and 18-in. depths totaling 78 cc. chloropicrin in 26 injections per site were planted in February with Elberta peach trees averaging minus 0.39-in. trunk diameters. The first season's average trunk diameter increases were 1.05 and 0.72 in. with root-knot-resistant and susceptible cover crops respectively. Nonfumed increases were only 0.60 and 0.47 in. per respective cover. After 5 seasons, average trunks on fumed sites with resistant cover measured 3.88 in. and limbs occupied 802 cu. ft. of spread. Trunks on nonfumed sites with susceptible cover measured 2.59 in. and limbs occupied 409 cu. ft. Including 1948 yields, average trees on fumed sites produced 46.4 lb. and 23.9 lb. fruit with resistant and susceptible cover respectively. Trees on nonfumed sites produced 18.2 lb. and 9.7 lb. fruit with respective covers. Average trees on sites fumigated deep and shallow with 210 cc. chloropicrin in 70 injections and grown with root-knot-resistant cover 4 seasons produced 1242 cu. ft. limb spread and 4.49 in. trunk diameters compared with 538 cu. ft. and 3.01 in. on nonfumed sites. Fumigating subsoil produced greater tree vigor than fumigating larger shallow areas. Similar results obtained with fig and grape.

The Status of Phymatotrichum Root Rot of Cotton in Louisiana in 1948. NEAL, D. C. Cotton root rot caused by the fungus *Phymatotrichum omnivorum* was first reported in Louisiana in 1947. At that time two infested localities were located in the Red River alluvial area. One is in Bossier Parish embracing two separate fields of approximately 30 acres, and the other in Caddo Parish consisting of about 4.5 acres. These two parishes were surveyed again during the past season and two new infested localities were found. One consists of only a small spot roughly 35×50 feet in Bossier Parish and located 1.5 mi. north of the original 1947 infested fields. The other is in Caddo Parish and consists of approximately 13 acres located about 8 mi. northwest of the first field found infested in 1947. The points of interest concerning the prevalence of the disease in Louisiana are that it is confined entirely to the Red River cotton area bordering Arkansas and Texas; and that among the 4 localities known to be infested, one small spot was found in 1948 which did not occur in 1947 or was overlooked when that survey was made. The occurrence of the disease in a fertile basin both to the east and west of Red River and in fairly close proximity thereto suggests the possibility of its introduction by floods.

Phytophthora cinnamoni and Ramularia sp. Pathogenic on Camellia japonica. PLAKIDAS, A. G. *Phytophthora cinnamomi* Rands and an apparently undescribed species of *Ramularia* have been repeatedly isolated from diseased roots of sickly or dying camellias. Two-year old camellias, var. Sarah Frost, with balled roots, were transplanted in 2-gal. crocks in nonsterilized compost soil infested with pure cultures of the two fungi, alone and in combination. *Ramularia* by itself proved mildly parasitic, causing a slight reduction in growth. In the Phytophthora series, 1 of 12 plants was killed; and in the series inoculated with both fungi, 4 of the 21 were killed. In each series, the remaining plants were sickly, making hardly any new growth during the season. Rooted camellia cuttings were planted in chloropicrin-sterilized compost soil infested with pure cultures of the two fungi separately and in combination. The roots of the cuttings were dipped in water suspensions of the fungi before planting. All the plants thus treated were killed within 2 weeks. A new lot of rooted cuttings was planted in the same soil, but without dipping their roots in fungus suspensions. By the end of the summer, 7 of 14 plants in the Phytophthora series, 11 of 20 in the Phytophthora + Ramularia series, and 1 of 14 in the Ramularia series were killed. All the check plants lived and grew vigorously. Of the surviving plants, those inoculated with both fungi were the least thrifty. A synergistic action between *Phytophthora* and *Ramularia* was apparent in both experiments.

Witch's Broom, a Graft-Transmissible Disease of Arizona Ash (Fraxinus berlandieri). PLAKIDAS, A. G. A disease characterized by yellowish leaves about $\frac{1}{4}$ to $\frac{1}{3}$ natural size and multiple, spindly, wirelike shoots was observed in September, 1943, on a tree about 20 years old on the Louisiana State University campus. Of six main branches, all rising from a common crotch, one was completely diseased to the top, four had scattered diseased shoots about halfway to the top, and one was normal. The diseased foliage persisted into late fall long after the healthy leaves were shed. Cuttings from the diseased tree failed to root; those from healthy trees rooted readily. Three young healthy trees in 12-in. pots were grafted in August, 1947, by in-arching diseased shoots, the cut ends of which were kept in water. Two of the grafts made union. The only symptom developing the first season was a pale-green color of leaves, compared with the dark green of the check. In the following season, however, the grafted

trees made only feeble growth, with weak shoots 1 to 3 in. long bearing yellowish small leaves. There was considerable dieback. One of the two grafted trees, after putting out vestigial growth with minute yellow leaves from several buds, died back to the ground. The check trees have grown to a height of 4 to 6 ft. Most of the original tree was dead by 1947 and it was cut down. This apparently lethal disease has been found on other trees of the same species.

Effect of Calcium on the Development of Cotton Fiber, A Preliminary Report. PRESLEY, JOHN T. Studies were made on the effect of varying amounts of calcium on the development of cotton fibers. Plants of the Stoneville 2B variety were grown in washed sand with nutrients added in the form of a complete nutrient solution. The amount of calcium in the nutrient solution was varied from 10 p.p.m. to 250 p.p.m. Fiber produced by the plants at different levels of calcium was analyzed for length, strength, fineness, maturity counts, X-ray angle, and D. P. (degree of polymerization). Plants with abundant calcium produced fiber that was longer, stronger, and higher in maturity count than that produced by calcium-deficient plants. The X-ray angles decreased as the calcium supply decreased.

Report of Sub-Committee Chairman on Verticillium Wilt for 1948. PRESLEY, JOHN T. Verticillium wilt is now known to occur over the entire Cotton Belt of the United States from the Atlantic to the Pacific. Losses from the disease, although variable from year to year in any one particular locality, appear to be constantly increasing. The continued increase and spread of Verticillium wilt in cotton has brought about an intensification of efforts to produce a Verticillium-wilt-resistant variety. Considerable breeding work is being done in an effort to introduce resistance to Verticillium wilt into commercially acceptable varieties, and, also, selections are being made from existing varieties in the hope of obtaining tolerant strains. Workers in California, Arizona, New Mexico, Oklahoma, Mississippi, and South Carolina are so engaged.

Types of Nematode Injury on Small-Grain Seedlings. ROSEN, H. R. On seedlings of oats, wheat, barley, and rye representing fall-sown crops, two different groups of symptoms associated with nematodes have been found in Arkansas, particularly in years when the summer and fall seasons were exceptionally dry. One group of symptoms appears to be associated with the typical root-knot nematode and primarily involves swellings, mainly on the lateral seminal roots. The tops of such infected seedlings consist of dull grayish-green leaves which gradually turn yellow, the seed leaves being usually the first to show marked chlorosis. Another group of symptoms consists of localized rotting of seminal roots, often of the subcoronal internode, and a yellowing of leaves. When this internode is rotted through before coronal roots appear, the seedling dies and in some fields a 10 per cent reduction in stand has been estimated as the result of such rotting. In most instances the seedling recovers as the coronal roots develop and the final grain yield may show little ill effect. However, there appears at times considerable reduction in available winter pasturage. G. Steiner has identified *Aphelenchoides*, spp., *Paraphelenchus* spp., and a number of other nematode species in such rotted seminal roots and coronal internodes, but no meadow nematodes.

Soil Fumigants for Controlling Fusarium Wilt and Nematodes of Cotton. SMITH, ALBERT L. DD (Dichloropropane and Dichloropropene) and Iscobrome D (23 per cent ethylene dibromide by weight) were compared in row applications at 3.5, 7, and 14 gal. per acre. The 3.5-gal. rate for DD increased the lint yield 45 per cent and controlled wilt satisfactorily. This rate appeared to be the most economical when used in combination with a resistant variety. Approximately 10 gal. of Iscobrome D gave comparable results. With planting and treatment performed simultaneously DD reduced emergence at all rates, but emergence was not affected by Iscobrome D. At the 14 gal. rate for DD, abnormal plant type and growth resulted, presumably from nitrogen stimulation as a result of treatment.

Histological Studies of Bacterial Blight Infection of the Cotton Plant. THIERS, HARRY D., and LESTER M. BLANK. Histological studies were made of bacterial blight infection of leaves and floral bracts of susceptible and resistant varieties of cotton. Vascular and mesophyll tissues possessing characteristic disease symptoms in different developmental stages were examined. The bacteria enter the leaf through the stoma and become massed in the intercellular spaces of the spongy mesophyll. Cell walls of the tissue are destroyed and the cellular contents liberated, producing watersoaked lesions on the lower surface of the leaf. The bacteria then attack the palisade cells and cause destruction of that tissue indicated by the appearance of similar lesions on the upper surface of the leaf. Bacteria were not observed in the xylem and phloem

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but were observed in the parenchymatous sheath surrounding these vascular elements. Floral bract tissue appeared to be more extensively damaged than leaf tissue. The bacteria occurred in greater numbers and the damage was more extensive in lesions on susceptible varieties than on resistant strains. However, the method of tissue destruction appeared similar in both types of host. No morphological barrier was observed which might restrict development of the disease, suggesting that resistance is probably due to physiological incompatibility of host and parasite.

Virus Diseases of Shallot in Louisiana. TIMS, E. C. There are at least two distinct virus diseases affecting the shallot in Louisiana. A virus known as aster yellows has been in the State for at least 10 years. Shallots affected with this virus have a bright yellow color that is distinct from that of other diseases. Aster yellows does not spread rapidly in the field, and is not a very serious disease at the present time. Yellow dwarf is another virus disease that was recognized somewhat later than aster yellows. It causes a mottling, curling, and twisting of the leaves along with marked stunting of the affected plants. This virus spreads rapidly in the field and causes serious losses. It is transmitted by aphids in the field, and is easily transmissible by artificial means. Yield tests for 3 years showed that yellow dwarf reduced the green weight of shallots from 25 to 30 per cent. Yellow dwarf is hard to control under Louisiana conditions. The growing of disease-free seed stocks in isolated areas is helping to reduce the amount of yellow dwarf.

Bottom-Rot of Celery Caused by Corticium vagum B. & C. WEBER, GEORGE F. The disease, difficult to detect in fields, appears on the basal leaflets and succulent petioles of mature celery plants growing in dense stands on wet, highly organic soils under continued rather high humidity. Margins of leaf blades first show the disease, becoming wilted, blackened, and wet. The disease progresses toward the midribs and larger veins. It usually appears on young leaflets, spreading toward the center of the bunch but seldom into the heart of the plant. Loss of foliage is not excessive and early symptoms of the disease are not obvious, except in certain instances where the fungus spreading from the blade of the leaflet involves the secondary petioles and reaches the main petiole, causing collapse of the entire leaf. At the soil line near the base of the petioles or stalks, brown, circular to oval, slightly sunken lesions up to 1 in. or more in diameter may be observed on either outer or inner surface. The epidermis over these brown areas is intact and free from obstructions except for a barely discernible thin net of closely adhering, intertwined, brown, septate hyphae. These hyphae extend over the surrounding noncolored stalk tissue and frequently terminate in sclerotium-like tufts. The brownish luster, caused by the hyphae, of the surface of the petioles as observed in sunlight on a plant removed from the soil is intense at the soil line, fading to white several inches above. At this zone, the sexual stage of the fungus developed in a compact net of white superficial hyphae. Basidia and basidiospores were observed. On potato-dextrose agar the fungus produced characteristic mycelium and sclerotia.

Factors Affecting the Efficiency of Peanut Seed Treatments. WILSON, COYT. Increases in emergence of peanuts resulting from seed treatment with a dust disinfectant depend upon quality of seed, type and dosage of disinfectant, and storage conditions between time of treatment and time of planting. Increased emergence was greater on poor quality than on good quality seed. Average emergence over a 5-yr. period on untreated, machine-shelled seed was approximately 60 per cent. Emergence of treated, machine-shelled seed was approximately 80 per cent. Emergence of untreated, hand-shelled seed was about 80 per cent. Treatment increased emergence to about 86 per cent. Significant increases in emergence were obtained from the following seed protectants, arranged in ascending order: Spergon (98 per cent tetrachloro-para-benzoquinone), Dow 9B (50 per cent zinc trichlorophenate), Phygon (98 per cent 2,3-dichloro-1, 4-naphthoquinone), Arasan (50 per cent tetramethyl thiuramdisulfide), 2 per cent Ceresan (2 per cent ethyl mercury chloride), and Ceresan M (7.7 per cent ethyl mercury p-toluene sulfonanilide). Dosage was more important with mercurials than with organic treatments. Increasing the dosage of Phygon from 3 to 6 oz. per 100 lb. of seed did not appreciably affect emergence, but increasing the dosage of Ceresan M from 1½ to 3 oz. per 100 lb. decreased emergence almost 20 per cent. When the dosage of Ceresan M was increased to 6 oz. per 100 lb. there was almost no emergence. Treatments were less effective in preventing deterioration of seed at summer temperatures than at winter temperatures.

REPORT OF THE SIXTH ANNUAL MEETING OF THE POTOMAC DIVISION OF THE AMERICAN PHYTOPATHOLOGICAL SOCIETY

The meeting was held in the auditorium of the U. S. Plant Industry Station, Beltsville, Maryland, February 23 and 24, 1949. Approximately 100 members were in attendance. Seventy members and guests attended the annual banquet at the conclusion of the first day's sessions, followed by an illustrated lecture on "Agricultural Potentialities of the American Tropics" by M. H. Langford. During the business session, members discussed their preferences as to time of meeting of the parent society.

Officers elected to serve during the ensuing year were: *President,* C. S. LEFEBVRE; *Vice-President,* C. E. COX; *Secretary-Treasurer,* J. B. DEMAREE; *Councilor,* PAUL R. MILLER.

J. B. DEMAREE, *Secretary-Treasurer*

ABSTRACTS OF PAPERS

Natural Control of the Destructive Sweetclover Weevil Sitona cylindricollis Fabr. by an Entomogenous Fungus Parasite. ALLISON, J. LEWIS. Recent findings indicate that a natural biological control has been effective in destroying the sweetclover weevil in certain endemic localities. An entomogenous fungus parasite has been discovered to be the causal agent in this control. The fungus has been identified as a member of the genus *Hirsutella* and represents a new species. No perfect or ascigerous spore stage of the fungus has been found in nature or induced under laboratory conditions. None of the entomogenous fungi have been heretofore reported as parasites of the sweetclover weevil. The fungus attacks only living weevils but does not produce its horn-like clavae on which the conidia are borne until after the insects die and become mummified. The adult is the only stage in the normal cycle of the weevil that has been found parasitized. A feature in the life history of the weevil makes it especially vulnerable to attack by its fungus parasite. There is only one generation a year. The newly-developed adults appear during the summer but they rarely mate and oviposit until the following spring. As the fungus parasite is most destructive during late summer and early fall, it parasitizes many weevils prior to mating and subsequent oviposition. As in the case of all entomogenous fungi which have been reported as effective agents in biological control, this fungus is no exception in its pattern of cyclic development. The fungus parasitizes and destroys some weevils each year but does not become an effective agent in control until the sweetclover weevil population in a given area attains a peak. At this stage the fungus, which has been gradually building up, spreads rapidly attaining epidemic proportions and in a short time reduces the weevil population to a bare minimum. The cycle for both insect and fungus parasite must then start anew.

Chrysanthemum Stunt. BRIERLEY, PHILIP, and FLOYD F. SMITH. Chrysanthemum stunt, first recognized in 1945, became generally prevalent in greenhouse chrysanthemums in this country and Canada in 1946. Affected plants are smaller than normal, leaves are smaller and paler green, flowers smaller, and in some bronze- and pink-flowered varieties the color is bleached. Graft transmission was 100 per cent successful in 1947, but 55 per cent of the control plants also developed the disease. Transmission trials made between January and May 1948 resulted in expression of symptoms in only 5 of 50 grafts in August. When the test plants were flowered again in December 1948, stunt appeared in all of the 50 plants grafted with stunt scions, all of 48 inoculated by leaf rubbing, 10 of 35 inoculated by *Rhopalosiphum rufomaculatum* (Wilson), in low and perhaps nonsignificant proportions from transfer of 4 other aphid species, and in 6 of 100 noninoculated controls. The minimum observed period for expression of symptoms following grafting is about 3½ months; a more common interval is 7 to 10 months, or in the second flowering period after inoculation. Control practices indicated are selection of healthy plants during two successive flowering periods, control of aphids, and precautions against handling hazards.

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Host-Parasite Interrelationships of Nematodes in Root Diseases. CHITWOOD, B. G. This is an extremely broad subject, brought forward with the idea that factors often not considered caused field observers and experimentalists to reach false conclusions. Briefly, the variables in nematode-plant interrelationships are as follows: (1) Inoculum: A. Nematode genus, species, variety or race, genotype. B. Quantity of inoculum. (2) Effluvium: A. Attractant. B. Repellant or Inhibitor (this is a chemical by-product of root growth). (3) Plant: A. Quality, *i.e.*, genus, species, variety. B. Quantity at a given time. (4) Conditioning factors, including climatological and soil conditions, their effects on plant quantity and effluvium and on the nematodes' reaction to these factors. (5) Hazards to nematode, such as environmental risks and natural enemies. (6) Invasion of plant: A. Enzymes (action on plant). B. Mechanical destruction. C. Plant response. (7) Parasite and host growth and nutrition: A. Ability of plant to feed nematode properly. B. Possible poisonous materials in plant. C. Materials withdrawn from plant by nematode, as to quantity and quality (possible differential absorption). D. Rate of reproduction of nematode (conditioning factors: food, temperature and heredity). (8) Subsequent invasions and subsequent nematode generations. (9) Secondary invaders. (10) Transmission of other organisms by nematodes. If any of the above ten variables is changed the entire interaction may be changed.

The Production of Internal Bark Necrosis of Apple in Sand and Soil Cultures. CLULO, GENEVIEVE. The disease was produced at the end of the first growing season by adding 64 or more p.p.m. manganese in the form of manganous sulfate to sand cultures. Two soils were used: one on which the disease always developed; the other from a location where Delicious had been grown for twenty years without becoming diseased. $MnSO_4$, H_2SO_4, or sulfur added to either soil increased the incidence and severity of the disease on bark formed the previous season, less intensely on the current season's bark. $(NH_4)_2SO_4$ and $NaNO_3$ added to the disease-producing soil increased the incidence and severity of the disease on the current season's bark with no apparent symptoms on the bark of the previous season. When $Ca(OH)_2$, $CaCO_3$, $MgCO_3$, MgO, or Na_2CO_3 was added to disease-producing soil in the greenhouse the development of the disease was prevented for the duration of the experiments (5 years). These alkaline reagents increased the soil pH to 6.3–7.5. Trees planted in the orchard in soil treated with $Ca(OH)_2$ have not become diseased during a 10-year period. Individual trees often vary widely in their ability to absorb Mn under identical conditions. A pronounced oedematous condition of the bark may accompany the typical pimply symptom and is usually associated with treatments which stimulate rapid intake of Mn.

An Undescribed Virus Disease of Honeysuckle. CORP, V. H. *Lonicera brachypoda* var. *foliis aureo-reticulatis*, propagated for ornamental purposes, has been found to be *L. japonica* infected with a virus. This virus has been reported graft-transferable by Woods and DuBuy (Phytopath. 33: 637–655. 1943). Recently the virus has been graft-transferred four successive times without decrease in severity or other change in symptom pattern to the green seedlings of the yellow-veined parent and to *L. japonica* collected in the field. Within 15 to 20 days after grafting, the veins of leaves of the stock appear yellow. Symptoms vary from the original when the field-collected *L. japonica* clone is used as grafting stock. Yellowing appears along veins in young leaves and, as the leaf matures, spreads to interveinal areas resulting in a mottled appearance. There is no evidence that this virus is seed-transmitted or spread in the field other than vegetatively.

Botryosphaeria and Diplodia Associated with Cankers on Linden and Redbud. DAVIDSON, ROSS W., HORACE V. WESTER, and MARVIN E. FOWLER. Several strains of *Botryosphaeria ribis* and one of *Diplodia theobromae* were isolated from large conspicuous cankers on *Tilia neglecta* in the parks of Washington, D. C. These cultures were tested by inoculation into stems of several species of linden. *T. neglecta* was most susceptible to the fungi, as evidenced by the number of successful inoculations and the size of cankers formed. The tests indicated that *T. cordata*, *T. americana*, *T. dasystyla*, *T. platyphyllos*, and *T. europaea* were less susceptible to the fungi. The large perennial type of cankers on *T. neglecta*, from which the cultures were obtained, have not been reproduced in these inoculation tests. A common and destructive canker of *Cercis canadensis*, in the vicinity of Washington, D. C., is believed to be caused by *B. ribis*. Cultures of this fungus from cankers on redbud and on linden caused cankers when inoculated into stems of *C. canadensis* and *C. chinensis* although the latter host is not known to be susceptible to natural infection by *B. ribis*. Check inoculations with sterile culture media in small stem wounds resulted in no cankers on any of the hosts.

Resistance of Lycopersicon hirsutum × L. esculentum Hybrids to Infection with Tobacco Mosaic Virus by Handling and Pruning. DOOLITTLE, S. P., and W. S. PORTE. Nearly all plants of segregating progenies of mosaic-resistant *Lycopersicon hirsutum* × *L. esculentum* crosses develop mosaic symptoms when inoculated with a yellow strain of tobacco-mosaic virus by leaf rubbing with use of carborundum powder. Many such plants, however, resist infection by means comparable to those causing natural infection in field and greenhouse. When inoculated (without carborundum) by lightly rubbing one leaflet with the virus diluted 1–10, 1–50, 1–100, 1–1000, and 1–10,000, series of ten young Marglobe plants were uniformly infected. Identical tests with three hybrid lines showed an average infection of 84 per cent at 1–10, 50 per cent at 1–50, 26 per cent at 1–100, 25 per cent at 1–1000, and 10 per cent at 1–10,000. When five lots of ten plants of each hybrid line were handled as in transplanting after first handling a mosaic plant, the average infection for all lines was 9 per cent and for Marglobe 66 per cent. When ten plants had one leaf pruned with a knife first used to prune a mosaic plant, the average infection in fifty plants of each hybrid line was 5 per cent and for Marglobe 40 per cent. When tops of ten plants were cut off with a knife carrying mosaic virus, forty plants of each hybrid line averaged 22 per cent infection and Marglobe 61 per cent.

Longevity of Some Pythium Species in Maize-Meal Agar Cultures. DRECHSLER, CHARLES. Many cultures of *Pythium* species grown in test-tubes on maize-meal agar containing some maize meal in suspension were successfully transferred 6½ to 7 years after inoculation. On adding sterile water to the substratum, which had been in an air-dry, horny condition for about 4 years, some cultures of *P. ultimum* and *P. oligandrum* showed normal internal organization in fully 95 per cent of the very large number of oospores present. Survival in lesser ratios was noted among oospores of *P. debaryanum, P. irregulare, P. mamillatum, P. vexans, P. salpingophorum, P. dissotocum, P. complens, P. acanthicum, P. periplocum, P. oedochilum, P. palingenes,* and *P. ostracodes.* On addition of sterile water 9¾ years after their inoculation, cultures of *P. Violae, P. oligandrum, P. acanthicum, P. oedochilum,* and *P. palingenes* showed living oospores of correct internal structure present in the resoftened agar, and transfer to fresh substratum resulted in renewed growth of these species. Some transfers from old cultures were unsuccessful, although oospores of correct living structure were present. In these instances presumably all surviving oospores were in a dormant condition, and would have required a period of after-ripening before becoming capable of germination. The culture of *P. Violae* used was isolated in May 1938 from softened cortex of diseased pansy roots dug up in Washington, D. C.

Natural Spread of Phony Disease to Apricot and Plum. HUTCHINS, LEE M., and JOHN L. RUE. Natural spread of phony disease to seedling trees of two species of apricot and three species of plum was observed at Fort Valley, Georgia. The species affected and the severity of symptoms under the cultural conditions provided were as follows: symptoms pronounced in common apricot (*Prunus armeniaca*); less marked in *P. hortulana* (Hortulan plum) and *P. mexicana* (big tree plum); highly indefinite in *P. mume* (Japanese apricot) and *P. angustifolia* (chickasaw plum). Proof of infection was established through indexing by root grafts to normal peach nursery trees. Where the root piece carried the virus, the index tree developed phony disease after an incubation period of 18 months. Infected trees of the latter two species were virtually symptomless carriers of the virus. The hazard of spread of phony disease from infected wild plums to near-by peach orchards is discussed.

Roadside Survey for Spot Anthracnose of Flowering Dogwood in Maryland, Virginia, and West Virginia. JEHLE, R. A., and ANNA E. JENKINS. In reporting the existence of a spot anthracnose of flowering dogwood (*Cornus florida*) and describing its pathogen (*Elsinoë corni*), Jenkins and Bitancourt (1948) included one locality in Maryland (Beltsville) and one in Virginia (Norfolk) as the known distribution of the disease. A survey for this spot anthracnose conducted during summer and autumn of 1948 reveals that in Maryland it is widespread and often severe in the southern part and that its prevalence and severity gradually diminish to the northward until it seems to disappear just above the 39th parallel. It was not detected at all in northern Maryland. It was found in two localities in Sussex County, southern Delaware and in three localities in Accomack County, on the Virginia Eastern Shore. In northern Virginia and northern West Virginia along route 50 from the District of Columbia to Allegheny Front no evidence of the disease was found.

Studies on the Overwintering of Monilinia fructicola in Peach Twig Cankers. KEPHART, JOYCE E., and JOHN C. DUNEGAN. As part of the studies on twig canker fun-

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gus sporulation, tissue fragments of overwintered peach twig cankers from Michigan were cultured from January to April 1948, and similar work started on material from Virginia in January 1949. *Monilinia fructicola* was recovered from 71 per cent of the Michigan cankers and 30 per cent of the Virginia cankers. Culture plates from the Virginia cankers had a large number of saprophytic fungi whereas the plates from the Michigan material usually were pure cultures of *M. fructicola.*

Johnson Grass Smut in Relation to Sorghum Culture. LEUKEL, R. W., and JOHN H. MARTIN. Smut commonly infecting Johnson grass (*Sorghum halepense* (L) Pers.) was found to be identical with *Sphacelotheca holci* identified in 1934 by H. S. Jackson on sorghum in Venezuela. As previously noted, Johnson grass smut differs from *S. cruenta* in that its spores are larger, darker, more echinulate, and shorter-lived. It differs also in its limited host range and in its severe stunting effect upon susceptible plants. Infection may be induced by seed inoculation with chlamydospores, or by applying spores to freshly cut stubble. The mycelium was found to travel down into the crown, up into other shoots or culms, and into and along the rhizomes to infect other more distant shoots. It is not considered a serious menace to sorghum because of its short-lived spores.

Controlling Late Blight by Spraying Potato Cull Piles with Sprout Inhibitors. MARTH, P. C., and E. S. SCHULTZ. Waste potatoes that harbor the late-blight fungus and are deposited on cull piles are sources for initial late-blight infection. Cull piles also harbor potato insect pests. Potato tubers were sprayed with sprout inhibitors, or dormancy treatments, as they were deposited on cull piles, to determine the effect of such treatments on sprout inhibition. In exploratory tests during 1944 to 1947 in the Beltsville greenhouse, applications made with the methyl ester of naphthaleneacetic acid, with 2,4,5-trichlorophenoxyacetic acid (2,4,5-T) and with the methyl, ethyl, butyl, and isopropyl esters of this latter acid, were outstanding in preventing sprouting on Green Mountain, Katahdin, and Sebago. In 1947 at Presque Isle, Maine, potato cull piles were treated by spraying each layer of tubers with a 2500 p.p.m. concentration of 2,4-D (acid) and of 2,4,5-T (acid). Only 2,4,5-T prevented sprouting. In further field tests made in Maine in 1948 with the methyl ester of naphthaleneacetic acid and with the butyl and isopropyl esters of 2,4,5-T at a spray concentration of 2500 p.p.m., sprouting was prevented and sprout growth on sprouted tubers was inhibited.

Quarantine Aspects of Prunus Virus Control. MCCUBBIN, W. A. Prunus virus control programs aim both to provide virus-free planting stock and to protect the resulting long-lived orchard from infection. Some control measures can be carried out well by the grower or nurseryman; others require special technical aid; and in a number of cases regulatory supervision is needed. Control methods include 1) bud-testing techniques; 2) marking and removal practices; 3) isolation arrangements; 4) vector suppression; and 5) treatments (when these are available). In all these activities regulatory powers are likely to be called on in varying degree and in combination with grower and technical efforts to secure program completeness, to provide official certification, to maintain isolation requirements, and to assist in vector suppression. Because of the nature of the problem, regulatory features must be undertaken largely by the State. Federal authority, effective in the foreign virus control field, is not adapted to deal with many phases of domestic control.

Variability in Monoconidial and Hyphal-Tip Isolates of Helminthosporium turcicum. ROBERT, ALICE L., and MERLE T. JENKINS. A wide range of virulence exists among monoconidial isolates of *Helminthosporium turcicum.* Pathogenicity of these isolates may change from one year to the next when grown on artificial media or when passed through a susceptible host. Twenty-five monoconidial isolates from each of two leaf lesions caused by two different monoconidial cultures differed pathogenically. Similar variation was obtained with ten hyphal-tip isolates from each of five single conidia. Some of the hyphal-tip isolates caused no infection. Growth of these isolates on potato-dextrose agar showed differences in cultural characters, rate of growth, and production of conidia. Of twelve non-spore-producing hyphal-tip cultures, ten produced no infection and two produced very slight infection.

Pulling vs. Spraying Potato Tops with Herbicides for Control of Virus Diseases. SCHULTZ, E. S. Pulling healthy potato tops August 1 in Aroostook County, Maine, before the plants are infested with viruliferous aphids, results in healthy seed potatoes. Recently potato tops were killed with herbicidal sprays to compare this method for control of virus diseases with the more laborious one of pulling the plants. In 1944 tests the tops were treated August 15 and the tubers harvested September 2. Pulling tops

resulted in 6 per cent leaf roll; Sinox spray, 14 per cent; and controls, 33 per cent. In 1946 tops were treated August 23 and tubers harvested September 6. Pulling tops resulted in 2 per cent mosaic; sulfuric acid spray, 2 per cent; Sinox spray, 4 per cent; and controls, 20 per cent. In 1947, tops pulled, sprayed with Sinox General, with Vine Kill, and controls, treated August 27 and harvested September 9, resulted in, respectively, 3, 4, 3, and 12 per cent mosaic. The results in 1944 indicate that the stalks remaining alive for two weeks after spraying with an herbicide transmitted some additional leaf roll to the tubers when aphids were numerous. In 1946 and 1947 the treated stalks alone did not transmit virus diseases when aphids were scarce. These results indicate that virus diseases can be controlled by killing the tops early in August with herbicides when the tubers are harvested a few days after the tops are killed.

Further Studies on Barley Powdery Mildew (Erysiphe graminis hordei). TAPKE, V. F. A previous investigator reported that, in greenhouse-grown barley plants, resistance to infection with powdery mildew varies directly with the age of the plant at the time of inoculation. The writer's results indicate that not necessarily the age, but rather the relative tenderness or toughness of the plant tissue as conditioned by environment, is the critical factor. At Beltsville, Maryland, five varieties of barley plants in the heading stage in the greenhouse proved highly susceptible when inoculated in December, January, and February, but infection progressively declined from March through the conclusion of the test in May. Evidently this was due to a gradual change from the lush, tender, susceptible tissue developed in the dim, short, cool days of December, January, and February to a tougher, less susceptible tissue developed in the longer and usually much brighter, warmer days accompanying the progress of the season during March, April, and May. This is in line with the writer's recently reported studies showing that the environmental conditions to which the plants are exposed before inoculation may materially influence their reaction to mildew after inoculation.

Growing Two Annual Greenhouse Crops Instead of One in Studies on Barley Smuts. TAPKE, V. F. Under the controlled conditions of greenhouse culture, exceedingly rapid progress can be made in studies on physiologic races of the covered and two loose smuts of barley, on varietal resistance to the various races of each smut, *etc.* The writer has developed a mass-production method of testing barleys for smut resistance whereby, in his allotted space, 61,568 plants are grown instead of the 9,190 plants that would be grown under methods commonly used in the past. Under the new intensive system, seed is germinated in small pans and seedlings are transplanted 1 in. apart in rows only 2 in. apart. The plants are lightly watered. This restricts growth, saves water and time spent in watering, and practically eliminates weeds and mildew. Studies have shown that in the greenhouse very small plants yield data as reliable as that from very large plants. Two crops are grown annually. The first is transplanted in the greenhouse September 10. Artificial light is applied 1 hr. daily, midnight to 1 A.M., from mid-October to heading in late November or early December. The second crop is transplanted in January. Under this system the field hazard of failure due to unfavorable conditions for infection is eliminated.

Studies on Selenium Therapy of Meadow Nematode-Infected Boxwood. TARJAN, A. C. Active adult meadow nematodes from infected boxwoods were immersed in solutions of 5 to 1000 p.p.m. of sodium selenate. None of the concentrations used had any noticeable effect on the nematodes. However, application of solutions of 25, 50, and 100 parts of sodium selenate per million of soil to the soil around roots of 12-yr.-old nursery-grown boxwoods, *Buxus sempervirens angustifolia* West and *B. sempervirens handsworthii* Dallimore, caused striking fluctuations in root nematode populations in contrast to the controls. Similar results were evidenced in populations of free-living nematodes in the soil around the roots of treated plants. A special sampling technique involving a 3-wk. immersion of nematized root samples in Baermann funnels was used in obtaining quantitative determinations of nematode populations in boxwood roots. Concentrations of 50 and 75 p.p.m. of sodium selenate powder were applied as a soil amendment around the roots of 13-yr.-old plants. Root samples were taken at bimonthly intervals for a period of 5 months. Resulting nematode counts for the selenium-treated plants were lower at the 1 per cent level of significance than nematode counts for the controls. Apparently selenium is ingested by the feeding nematodes and a toxic reaction results.

Following are titles of other papers presented at the meeting of the Potomac Division of which abstracts were not submitted for publication:

Rhizoctonia solani, a Destructive Pathogen of Alta Fescue, Smooth Brome Grass and Birdsfoot Trefoil. J. LEWIS ALLISON, HELEN S. SHERWIN, IAN FORBES, JR., and ROBERT E. WAGNER.

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Cultural Characteristics of Several Species of Trametes. ROSS W. DAVIDSON and FRANCES LOMBARD.

Effective and Parasitic Strains of Rhizobium. S. W. ERDMAN.

An Undescribed Species of Periconia on Sorghum. C. S. LEFEBVRE, A. G. JOHNSON, and HELEN S. SHERWIN.

Agricultural Potentialities of the American Tropics. M. H. LANGFORD.

Incidence of Fusarium Infection as Affected by Root-Knot Nematodes. W. D. MCCLELLAN and J. R. CHRISTIE.

A Review of 1946–48 Weather Conditions in Relation to Late Blight Phytophthora Infection. MURIEL O'BRIEN.

A New Disease of Pin Oak, Possibly Caused by the Nematode Hoplolaimus coronatus Cobb. R. M. VIGGARS and A. C. TARJAN.

Pathological Observations in the Low Countries. W. H. WHEELER.

PHYTOPATHOLOGICAL NOTES

Root Inoculation, a Method Insuring Uniform Rapid Symptom Development of Bacterial Ring Rot of Potato.—In greenhouse or field studies with bacterial ring rot of potato caused by *Corynebacterium sepedonicum* (Spieck. and Kotth.) Skapt. and Burkh., the need arises for a reliable, efficient method of inoculation. With previously employed methods, such as hypodermic injection in the sprouts, needle puncture in the eyes, or momentary dips in bacterial cultures, infection and resultant symptoms do not always occur. Frequently only one stem of a plant becomes infected while another from the same seed piece remains healthy; or infected plants may develop symptoms only unilaterally.

The fact that these methods afford entrance of the agent into only a few of the many vascular elements of the plant perhaps accounts for the irregular development of symptoms. Larson,[1] working with tomatoes, obtained ring-rot symptoms in plants transplanted to soil freshly inoculated with ring-rot organisms, indicating that invasion occurred through damaged roots. Tyner[2] demonstrated that potato plants could be infected readily by dipping wounded root tips in a bacterial suspension, although he obtained negative evidence of the occurrence of field spread by row irrigation water.

With a view to determining the degree of uniformity of infection and the rate of symptom development in root inoculated plants, 10 seed pieces of Bliss Triumph potatoes were sprouted and rooted in moist peat moss for 20 days at 20° C., at the end of which period 1- to 2-in. sprouts and 2- to 4-in. roots had formed. These plants were removed from the peat moss, the roots were washed in running water, and the majority of roots were cut with scissors ¼ in. from their tips. Cut roots of 10 plants were dipped for 10 sec. in a water suspension of infected tissue expressed from the vascular ring of a ring-rot tuber. Two plants with cut roots, not so dipped, were retained as controls. Immediately following inoculation all seed pieces were planted in moist soil in 6-in. porous clay pots and placed at 22° C. Plants were observed daily in order to detect the first symptoms of ring rot.

Ring-rot symptoms developed rapidly and with unusual uniformity in 32 to 38 days following inoculation. The average number of days elapsing from inoculation to first expression of symptoms was only 35, which is a much shorter period than the 50 days at 22° C. air temperature or 44 days at 22° C. soil temperature, which were determined in other experiments to be required by plants hypodermically sprout-inoculated. Of greatest interest was the fact that all root-inoculated plants became infected and developed disease symptoms very rapidly and uniformly fol-

[1] Larson, R H. The ring-rot bacterium in relation to tomato and eggplant. Jour. Agr. Res. [U. S.] **67**: 309–325. 1944.

[2] Tyner, L. E. Studies on ring-rot of potato caused by *Corynebacterium sepedonicum*. Sci. Agr. **27**: 81–85. 1947

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lowing the earliest symptoms on the lower leaves. Control plants remained healthy. At harvest, 62 days after planting, all leaves of inoculated plants had developed typical ring-rot symptoms including severe wilt.

In a complementary experiment the quantitative aspect of root inoculation was investigated. Twenty seed pieces were sprouted and rooted in peat moss for 20 days at 20° C. After this period 15 to 20 roots had formed. Following removal from the moss the pieces were washed thoroughly in running tap water and divided into 6 lots of 4 each. All the roots in the first lot, $\frac{1}{2}$ of the roots in the second, $\frac{1}{4}$ of the roots in the third, 2 roots in the fourth, and no roots in the fifth lot were severed $\frac{1}{2}$ in. from their tips and dipped for 10 sec. in a water suspension of ring-rot diseased tuber tissue. A control lot in which roots were neither cut nor dipped was included. All seed pieces were planted in moist soil in 6-in. porous clay pots and retained at 22° C. All plants were observed daily for first ring-rot symptoms and were harvested 51 days after planting, when smears of all stems were made at a point 1 in. above the soil and were stained by the modified Gram stain method of Racicot, Savile, and Conners.[3]

Root inoculation resulted in 100 per cent infection followed by early uniform ring-rot symptoms in 26 days in all plants which had at least $\frac{1}{4}$ of their total number of roots cut and inoculated. In 3 of the 4 plants having only 2 roots cut and inoculated, symptom development was somewhat slower (29 days); also, one plant of this lot failed to develop symptoms although ring-rot organisms were detected in the stem. On the plants whose roots were not cut previous to dipping in the bacterial suspension, no symptoms were expressed during the 51-day period; however, 2 of the 4 plants were harboring moderate numbers of the causal organism in the aerial stems as shown by Gram-stain diagnosis. It is logical to assume that minor root damage occurred during the planting operation and provided entrance for small numbers of bacteria which were, however, too few to cause symptoms to be expressed. Plants in the control series remained healthy and carried no Gram-positive bacteria at harvest. Field studies at Lincoln in 1947 confirmed these greenhouse results.

To summarize, the root-inoculation method was found to be a most efficient technique for effecting ring-rot establishment and rapid uniform development of the disease in the potato plant. Also the number of infection courts, within limits, had little influence upon the rapidity or severity of ring-rot symptomatology since as few as 2 roots provided entrance for enough bacteria to produce disease symptoms of average severity.—ARDEN F. SHERF, Department of Plant Pathology, University of Nebraska, Lincoln, Nebraska.

[3] Racicot, H. N., D. B. O. Savile, and I. L. Conners. Bacterial wilt and rot of potatoes; some suggestions for its detection, identification, and control. Amer. Potato Jour. 15: 312–318. 1938.

Relative Effects of Septoria lycopersici and of Possible Gaseous Emanations from Ripe Fruit on Defoliation of Tomato.—It is a common observation that defoliation of tomatoes occurs more rapidly on plants bearing a heavy load of fruit than on plants with few fruits or in the vegetative condition. No entirely satisfactory explanation has been offered. Samson[1] has demonstrated, in sand culture, that available nitrogen may be a factor, although he could not detect significant differences between fertilizer treatments in the field. Skok[2] conducted limited experiments both in the greenhouse and in the field and concluded that "it is probable that gaseous emanations from ripe fruits present on the vines are a major contributing factor resulting in defoliation of tomato plants as grown under commercial field culture. On the basis of what is known about such emanations from ripening fruits of various kinds, and on additional experimental evidence, ethylene may be one of the principal compounds involved." Since

TABLE 1.—*Defoliation of tomato as affected by fruition and Septoria lycopersici*

Determinations	Treatment							
	Non-dusted				Dusted			
	Fruit added	Normal fruit	Picked pre-ripe	Deflorated	Fruit added	Normal fruit	Picked pre-ripe	Deflorated
Av. length of branch; inches	44.8	52.1	48.9	63.1	48.3	48.1	43.8	58.2
Av. portion of branch defoliated; inches	35.4	42.4	39.1	41.1	29.2	29.7	27.4	28.0
Av. per cent defoliated	79.0	81.4	79.9	65.1	60.5	61.7	62.6	47.8
Av. no. leaves lost per branch	18.6	21.0	20.0	19.4	14.7	15.2	14.5	14.8

it had been possible to cause almost complete defoliation of seedling plants by inoculation with *Septoria lycopersici* Speg. in the greenhouse, without the presence of fruiting plants, it seemed desirable to devise a field experiment to check Skok's hypothesis.

In 1943 two adjacent plots were set with Indiana Baltimore, a variety highly susceptible to *Septoria lycopersici.* The plants were infected when set in the field, June 4, and weather conditions favored development of the disease throughout the season. Each plot consisted of 4 rows of 10 plants each. In each plot, (1) one row was kept completely sterile by removing all flower buds; (2) in one row the fruits were picked just as they began to turn pink; (3) in one row the fruits were allowed to ripen normally; and (4) in one row the fruits were allowed to develop normally but additional ripe fruits, equal to the normal load, were placed under the vines. As soon as the plants began to flower, one plot was kept dusted throughout

[1] Samson, R. W. Defoliation of deflorated, late-set and variously fertilized tomato plants. Purdue Univ. Agr. Exp. Sta. 53rd Ann. Rept. 1940: 41–42.

[2] Skok, John. Defoliation of tomato plant as a response to gaseous emanations from the fruit. Bot. Gaz. **104**: 486–489. 1943.

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the season with a copper fungicide, while the second plot was left untreated. No attempt was made to measure the quantity of dust, and applications were made coincident with rains or heavy dews, the purpose being to maintain dust coverage. On September 15, using 3 main branches of each plant as a sample, the average length of branch, the average length and percentage defoliated, and the average number of leaves defoliated per branch were recorded for both the dusted and the non-dusted plot. These data are recorded in table 1.

Statistical treatment of the data summarized in table 1 shows a highly significant difference in defoliation between dusted and non-dusted plants with no significant difference in defoliation between all other treatments. The percentage of defoliation was relatively constant in each plot, irrespective of treatment except that on a percentage basis, deflorated plants defoliated less. However, on the basis of actual leaf drop the deflorated plants did not differ from plants with fruit. Dusted plots retained about 20 per cent more foliage than non-dusted plots. This experiment demonstrated that under epidemic conditions, *Septoria lycopersici* is a major cause of defoliation.

It is recognized that the physiological condition of the plant may influence the extent of defoliation but the importance of gaseous emanations by the fruit as a major cause of defoliation was not demonstrated in this experiment.—GEORGE B. CUMMINS, Department of Botany and Plant Pathology, Purdue University Agricultural Experiment Station, Lafayette, Indiana.

An Epizootic of Phyllocoptruta oleivora (Ashm.) on Citrus in Florida.—One of the most serious insect pests of citrus in Florida is the rust mite, *Phyllocoptruta oleivora* (Ashm.), which inflicts injury to the epidermal cells of the fruit and gives the rind of the fruit a brown, rusty appearance. This mite usually reaches the point of maximum infestation during late June and early July, shortly after the beginning of the rainy season. At such times, rust mite populations may be so high that mites will be present on virtually every fruit and leaf. Single fruits may be so heavily infested that the mites plus their cast skins give the fruit a dusty appearance. Yothers and Mason[1] reported that following such a period of maximum infestation on unsprayed trees the mites disappeared very rapidly, and that by the middle or end of September a diligent search was required in order to find even single specimens.

During October, 1948, an epizootic of rust mites occurred at Lucerne Park, Florida, in an unsprayed control plot consisting of 30 grapefruit trees. The rust mite population remained stable and at high levels through July and August. In September the mites began to disappear, and in October the epizootic was at its height. Figure 1 shows that be-

[1] Yothers, W. W., and A. C. Mason. The citrus rust mite and its control. U. S. Dept. Agr. Tech. Bul. 176. 1930.

tween August and October the percentage of fruit infested decreased from 98 to 20. In November there was a further decrease in the percentage of fruit infested.

Examination of the fruit showed that normal or healthy, as well as dead, rust mites were present. A high percentage of the living mites were dark yellow to tan, rather than the light lemon-yellow typical of healthy rust mites. These off-color mites generally were sluggish in their movements. The dead rust mites were tan to light brown. The appearance of the abnormal mites corresponded with the descriptions by Speare and Yothers.[2]

Rust mites which were alive but definitely abnormal in color and sluggish in their movements were mounted on slides in lactophenol and studied under the compound microscope. These rust mites were partially or completely filled with endoparasitic hyphal bodies. The means of infection is

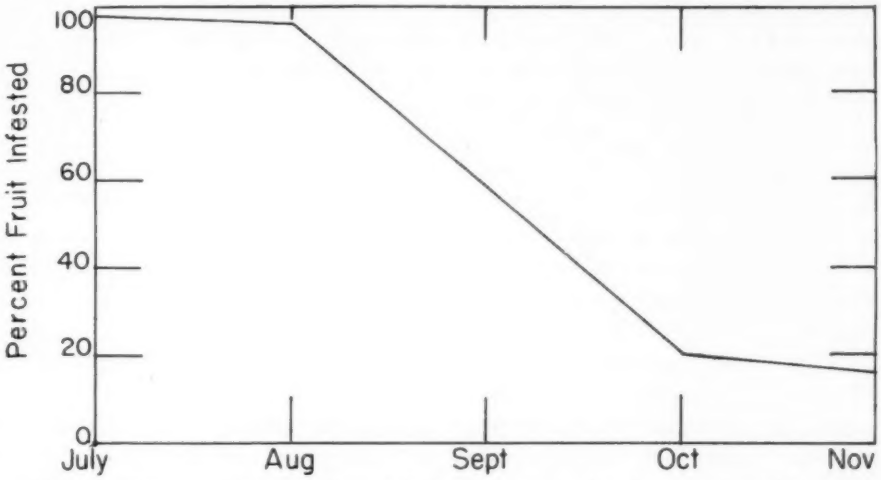

FIG. 1. The percentage of citrus infested with rust mites in an unsprayed control plot. Lucerne Park, Florida.

unknown. No hyphal bodies were found in normal rust mites of bright lemon-yellow color.

As yet the identity of the endoparasitic hyphal bodies in living mites has not been determined, but one fungus consistently associated with dead mites has been tentatively identified as a species of *Hirsutella* Pat. In mites which were dead, mycelia were found growing from the interior through both the anterior and posterior portions of the mites. In some specimens, which apparently had been dead for a long time, lateral emergence of mycelia was also observed. However, anterior and posterior emergence of 1–4 mycelial strands was typical. Under a hand lens, these mycelia appeared as silver threads. Throughout October and November the fungus fruited abundantly. At regular intervals along the mycelium,

[2] Speare, A. T., and W. W. Yothers. Is there an entomogenous fungus attacking the citrus rust mite in Florida? Science 60: 41–42. 1924.

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flask-shaped phialides were produced which were terminated by one or two long, filiform sterigmata, each bearing one spore. It remains to be proved whether or not this fungus actually originated from the endoparasitic hyphal bodies found in the living mites.

Because spore production was meager in his specimens, Speare[3] believed that the fungus spreads from mite to mite by means of the external mycelia. If *Hirsutella* sp. originates from the endoparasitic hyphal bodies, then infection of the rust mites is probably by other means.—F. E. FISHER, J. T. GRIFFITHS, JR., and W. L. THOMPSON, University of Florida, Citrus Experiment Station, Lake Alfred, Florida.

Some Studies on Downy Mildew of Millet.—Difficulties encountered in testing breeding materials of millets for resistance to downy mildew (*Sclerospora graminicola* (Sacc.) Schroet.) necessitated a series of experimental inoculations at Peiping, China. Three methods were tested in the greenhouse in 1947: a vacuum method by which surface sterilized seeds of millet were placed in a suspension of oospores of the fungus for 15 minutes and subjected to partial vacuum, a heavy spore load method by which moistened millet seeds were thoroughly covered with oospores of the fungus, and a dehulling method by which oospores of the fungus were mixed with the millet seeds after the hulls were removed. With the vacuum method, 19 per cent of the plants were infected; with the heavy spore load, 55 per cent. The dehulling method was not a valid test because other pathogens attacked the seed before it germinated. There was 2 per cent downy mildew infection in control plants that had not been inoculated.

Two other methods, both unsatisfactory, were tried: application of oospores to the plumule and root of germinated seeds during the first five days after germination, and planting in soil infested with oospores. There was only 8 per cent infection when the inoculation was made 1 day after germination as compared with 15 per cent infection when inoculation preceded germination of the seed. Within 2 days after germination the plumule and root were longer than 3 mm., and inoculum placed there then or later did not cause infection. When seed was placed in infested soil one day after seed germination, only 7 per cent of the plants were infected as compared with 14 per cent infected when non-germinated seeds were planted in infested soil. Controls for these two types of inoculation had no downy mildew.

Naturally infested soil serves as a source of inoculum, but the infection resulting may vary somewhat according to date of planting. Surface disinfected seeds were planted in infested soil taken from a field in which there had been about 20 per cent downy mildew on the millet. There was 7 per cent infection in the planting of December 1 and 6 per cent in that of January 12; but 12 per cent in the February 25 planting and 29 per cent in that of March 31. All controls remained free of disease.

[3] A. T. Speare. Unpublished manuscript.

Temperature affects the development of downy mildew. Millet seeds were inoculated with a heavy load of oospores and were germinated for two days at 16°, 20°, 24°, 28°, 32°, or 36° C., after which they were planted in soil in pots and placed in greenhouses at 25°–28° C. or at 20°–24° C. At the higher greenhouse temperature the plants headed within 63 days and downy mildew infection was determined then; but 97 days were required at the lower greenhouse temperature. A range of temperature between 24° and 32° C. during the first 2 days was most conducive to development of mildew even though the temperature might fall to 20°–24° C. after the first two days (Table 1). When the temperature was as low as 16° C. during the first two days, not more than 9–11 per cent of the plants were infected.

It has been customary to determine susceptibility of millet varieties by counting the whitened or shredded plants and plants with leafy ears at heading time. It is possible, however, to determine infection earlier by

TABLE 1.—*The development of downy mildew in millet inoculated with oospores and germinated at various temperatures for 2 days before transfer to greenhouses*

Temperature during first 2 days after inoculation	Percentage of infection developed during	
	97 days at 20°–24° C.	63 days at 25°–28° C.
16° C.	9	11
20° C.	16	19
24° C.	40	28
28° C.	34	43
32° C.	60	22
36° C.	33	16

inducing conidial sporulation on younger plants. Conidia form readily on the dorsal leaf surfaces if infected plants are placed in a saturated atmosphere at approximately 24° C. for about 12 hours or overnight. The percentages of infection determined by this method for seedlings of various ages were as follows: 20 per cent at 1-leaf stage, 62 per cent at 2-leaf stage, 17 per cent at 4-leaf stage, and 5 per cent at 7-leaf stage. Obviously, the method should be used when seedlings are in the 2-leaf stage of development. When this method of determining infection was compared with the former practice of determining infection on mature plants, a correlation coefficient of 0.9 was observed in 35 experiments. Inducing the formation of conidia makes possible a considerable saving in time and a testing in the greenhouse during the winter.—MU-HWA PU and TZAN-MING SZU, Peiping Agricultural Experiment Station, National Agricultural Research Bureau, Peiping, China.

Reactions of Wheat Varieties to Stripe Rust, Leaf Rust, and Stem Rust In Peiping, China.—As preliminary to the scientific breeding of rust-resistant wheats for northern China, 787 domestic varieties of wheat collected

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from 22 provinces of China and 656 foreign varieties were tested for their reactions to stripe, leaf, and stem rusts in Peiping in 1947–1948. The domestic collections included 651 farmer's varieties and 136 improved strains from various agricultural experiment stations and colleges. The foreign varieties included common and club wheats, durums, and emmers, and were introduced from the United States, various European countries, or Japan.

Many of the varieties were tested in the greenhouse and all were tested in field nurseries at Peiping. Inoculum of the three rusts, stripe rust (*Puccinia glumarum* (Schmidt) Erikss. and Henn.), leaf rust (*P. rubigo-vera tritici* Erikss. and Henn.), and stem rust (*P. graminis tritici* Erikss. and Henn.), was collected in the vicinity of Peiping in 1947. The physiological races comprising the inoculum were not identified, but according to unpublished data of H. R. Wang, race 123 of leaf rust and race 17 of stem rust are prevalent in that area. The most prevalent races of stripe rust are not yet known. Severe epidemics of the three rusts were induced in the nurseries by inoculating susceptible border rows with spore suspension by means of a hypodermic syringe. The fields were irrigated several times during the growing season to facilitate the spread of rust infection from border rows to the experimental varieties.

Most of the domestic Chinese wheats are susceptible to the three rusts. Of the 651 winter and spring varieties obtained from farmers, only four (1 from Hopei, 3 from Shantung) were resistant to stripe rust, only five (1 from Hopei, 2 from Shantung, 1 from Kansu, 1 from Kiangsu) were resistant to leaf rust, and only three (1 from Shantung, 1 from Kansu, 1 from Chahar)were resistant to both stripe and leaf rust. Not one was resistant to stem rust. Among the 136 improved Chinese varieties, 103 were highly susceptible to all three rusts, 13 were resistant to stripe rust, 14 were resistant to leaf rust, and 1 was resistant to stem rust. Four of the improved varieties, although susceptible in the seedling stage to stem rust, were resistant to all three rusts in the field. They were hybrids (No. 889, 918, 920, and 923) produced from a cross (made at the National Agricultural Research Bureau in Peiping) between a Chinese wheat and Percival's *Triticum albidum*.

The Chinese 166 wheat, which other workers have found resistant to many European races of stripe rust, was susceptible to stripe rust at Peiping.

Among the foreign introductions, numerous varieties of winter wheat were highly resistant to the three rusts. Resistant winter wheats from the United States were Cheyenne (C. I. 8885), Oro (C. I. 8220), Ioturk (C. I. 11388), Kanred (C. I. 5146), Poole or California Red (C. I. 3488), Turkey (C. I. 1558), Comanche (C. I. 11673), Tenmarq (C. I. 6936), and several hybrids from Kansas. Knoppies wheat from South Africa, Buisson from France, Almond from Sweden, Weizen Montcalm 245 from Switzerland, and Agr. No. 8 from Japan were highly resistant. Three winter wheats from Yenching University but of unknown origin also were highly resistant.

Hybrids originating from such crosses as Hope × Turkey, Comanche × Mediterranean-Hope, Oro × Mediterranean-Hope, Thorne × Mediterranean-Hope, or Mediterranean-Hope × Nebred were very susceptible to stem rust, both in the greenhouse and in the field.

Many of the spring wheats among the foreign introductions also were resistant to the three rusts at Peiping. Among them were Thatcher (C. I. 10003), Pilot (C. I. 11945), Merit (C. I. 12036), Marquillo (C. I. 6887), Marvel (C. I. 8876), Newthatch (C. I. 12328), Federation (C. I. 4734), Regent (C. I. 11869), and several hybrids from Minnesota. Several hybrids with Ceres-Hope-Florence × H44-Thatcher parentage were susceptible in the seedling stage to stem rust, but were moderately resistant in the field.

The variety Hope (C. I. 8178), which has long been highly resistant to or almost immune from many of the common races of stem rust in North America, was susceptible in the seedling stage and moderately susceptible in the adult stage to stem rust at Peiping. It was practically immune from stripe rust and highly resistant to leaf rust.

Triticum monococcum L., *T. Timopheevi* Zhuk., two varieties of *T. durum* Desf. (Acme, C. I. 5284, and Golden Ball, C. I. 6227), and one variety of *T. dicoccum* Sch. (Vernal, C. I. 3686) were highly resistant to or immune from the three rusts. Many varieties of *T. durum*, *T. dicoccum*, *T. spelta* L., *T. persicum*, *T. pyramidale* Mihi, *T. compactum* Host., and *T. macha* were resistant to the stripe and leaf rusts but were susceptible to stem rust. *T. sphaerococcum* Mihi and *T. orientale* Mihi were resistant only to stripe rust; and *T. turgidum* L. was resistant to none of the three rusts.

There seems to be little chance of obtaining the character of rust resistance by selection within the native wheat varieties of China. A breeding program based on the hybridization of Chinese wheats with wheats of foreign origin offers the best promise for securing rust-resistant varieties. The foreign wheats themselves are not entirely satisfactory because they generally mature 7 to 20 days later than Chinese wheats. In the winter wheat area of China, where stem rust usually is less severe than either stripe rust or leaf rust, it may be possible to use breeding materials that have resistance to the last two rusts. In the spring wheat area, where race 17 of stem rust is prevalent, some of the highly resistant Minnesota varieties may be used as parental material.—MU-HWA PU and OU-YANG YAO, Peiping Agricultural Experiment Station, National Agricultural Research Bureau, Peiping, China.

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ANNOUNCEMENT

ANNUAL MEETING—1949

The forty-first annual meeting in New York City is tentatively scheduled for December 28, 29, and 30, 1949. To meet program publication deadlines set by the AAAS, the Secretary must have by October 5, *TITLES* of papers to be presented. No titles will be included in the AAAS general program if received by the Secretary of the APS after October 5. *ABSTRACTS* will be accepted by the Secretary until October 15.

The Abstract Committee may reject abstracts even though titles have been forwarded to the AAAS for inclusion in the general program. To avoid embarrassment authors are urged to make certain that the material to be presented meets the requirements of the Society and to give careful attention to the preparation of abstracts.

Projection costs will amount to about $25.00 per session for one lantern and operator. If two lanterns are required in a session it will cost $10.00 more. At this rate our projection costs are estimated at $425.00 if we have one lantern per paper reading session. The Society can save at least $170.00 by using slides of one size only. It seems that kodachrome is the preferable size because of the wide use of color. In planning and preparing material for presentation, authors are earnestly solicited to use only 2×2 slides, and thus to help keep down costs of the meeting. The Secretary of the AAAS is attempting to work out a plan by which a part of the costs for projection can be rebated by the AAAS to the participating societies. The amount of the rebate will be based upon membership, attendance, and registration with the AAAS.

Final arrangements for joint sessions with other societies, symposia, etc., must be completed by September 15, 1949, in order that meeting room assignments can be made; meeting rooms will be at a premium and it will be very difficult to change or increase the requirements after assignments have been made. Please let the Secretary know of your needs as soon as possible.

CURTIS MAY, *Secretary*
Plant Industry Station,
Beltsville, Maryland